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CANE SUGAR AND POTASSIUM NITRATE
AS
PLASMOLYSING AGENTS

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CANE SUGAR AND POTASSIUM NITRATE
AS
PLASMOLYSING AGENTS

THESIS

PRESENTED AT THE

UNIVERSITY OF FRIBOURG, SWITZERLAND

TO THE

FACULTY OF MATHEMATICS AND NATURAL SCIENCES

TO OBTAIN

THE DEGREE OF DOCTOR

BY
WILLIAM A. BECK
OF
PITTSBURGH, PA. U. S. A.

TO MY MOTHER

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INTRODUCTION

Solutions of cane sugar and potassium nitrate have been employed since DE VRIES introduced the plasmolytic method of studying the osmotic relations in plant tissues in 1877 (**32** p. 8).

DE VRIES thought potassium nitrate and sodium chloride were better suited as agents than cane sugar (**32** p. 86). WIGGINS used calcium chloride in preference to potassium nitrate, "because of the tendency of the latter to make the protoplasm more permeable" (**33** p. 32). URSPRUNG and BLUM, after employing potassium nitrate for years, preferred sugar solution (**27** p. 90). STEINBERGER used potassium nitrate and sodium chloride, avoiding the use of sugar, because she suspected that with the extraction of water from the guard cells, starch is regenerated (**23** p. 4).

Before entering upon a series of osmotic studies, I determined the reliability of cane sugar and potassium nitrate as agents. The results of these preliminary tests grew beyond the limits of an introduction, and were made the subject of this publication.

This work was carried out at the University of Fribourg, under the direction of Professor ALFRED URSPRUNG, to whom I wish to express my thanks.

TERMS

The terms were chosen according to the system employed by URSPRUNG and BLUM (cf. **29**). The symbol O_g is employed to express the osmotic value at incipient plasmolysis. $O_g(C_{12}H_{22}O_{11})$ is the O_g value when cane sugar is the agent, and $O_g(KNO_3)$ is the O_g value when potassium nitrate is the agent. **i** Represents the osmotic coefficient.

PRELIMINARY CONSIDERATIONS

Before deciding upon the modes of testing, the following considerations were made:

Consideration I: When the O_g of a cell or tissue, is determined by the plasmolytic method, water necessarily passes from the cell or tissue into the agent.

Consideration II: Whenever water is extracted from the cell, the molecular constitution of its contents may not change, or it may vary as the result of: (a) physico-chemical causes; (b) the stimulation of enzymatic activities; (c) the physiological activity of the protoplasm. The extraction of water cannot be avoided, but the period of immersion in the agent can be reduced to the minimum of time required to produce plasmolysis. It is probable that, other things remaining the same, any induced change, due to the extraction of water, will be approximately the same in equal periods of time, for the measurements on a given tissue, and the results would be comparable.

Consideration III: MORSE was unable to set up a satisfactory table of equivalent pressure values for potassium nitrate solutions, because the membrane of the osmometer deteriorated in the electrolyte, and became permeable (**20** p. 92 and 209). He found that sugar solutions under test, maintained a constant pressure for 60 days. If the protoplasmic film of the living cell should become affected in such a way by the potassium nitrate, that the salt could enter the cell, evidently the O_g-reading would be false.

Consideration IV: If an agent that has entered the cell induces a molecular change in its contents, by enzymatic or other activity, a source of error of unknown magnitude is introduced.

Consideration V: Other things remaining the same, the time that is necessary to produce incipient plasmolysis is a function of the diffusion rate of the agent.

The diffusion rates of potassium nitrate and cane sugar were determined by THOVERT (**15** Vol. I p. 246-248). His results are expressed according to the formula

$$dS = -k \cdot q \cdot \frac{dc}{dx} \cdot dt \cdot 10^3$$

in which dS is the quantity of solute which passes the point x, in the time dt, through the cross-section (area in cm^2) of the diffusion cylinder,

under the influence of a concentration difference $\frac{dc}{dx}$. The constant k depends upon the temperature and the concentration of the solution. His data are as follows:

Potassium Nitrate			Cane Sugar		
Concentration in mol	Temperature in C	k cm^2/Days	Concentration in mol	Temperature in C	k cm^2/Days
3·90	17·6°	0·89	1·97	18·5°	0·13
1·40	17·6°	1·10	0·97	18·5°	0·24
0·30	17·6°	1·26	0·30	18·5°	0·31
0·02	17·6°	1·28	0·005	18·5°	0·33

At the concentration 0·3 mol the speed of diffusion is about one fourth as great for the sugar as it is for the potassium nitrate. A solution of sugar, that is isosmotic with a 0·3 mol potassium nitrate solution, is more concentrated than 0·3 mol; consequently the diffusion rate of the sugar solution that is isosmotic with the 0·3 mol potassium nitrate solution, has a diffusion rate that is less than one fourth as great as that of the equivalent potassium nitrate solution (since the diffusion rate decreases with the concentration).

Under this consideration potassium nitrate is a more favorable agent than sugar (cf. 32 p. 11).

Consideration VI: STEINBERGER rejected cane sugar as a suitable agent for the study of O_g variations in guard cells, on the claim: "Rohrzuckerlösung dagegen veranlaßt, infolge ihrer stark wasserentziehenden Eigenschaft und geringen Fähigkeit in das Plasma einzudringen, eine Regeneration der Stärke in den Schließzellen und führt so in kurzer Zeit eine weitgehende Herabsetzung des osm. Wertes herbei (23 p. 4)". This corresponds to the difficulty referred to in the second consideration, but her data do not prove her contention. On pages 4 and 27 she says $O_g(C_{12}H_{22}O_{11})$ was 0·6 mol after 5 minutes immersion, 0·2 after 15 minutes immersion, and 0·2 mol after 60 minutes immersion. The sugar diffuses very slowly (cf. tables 11 and 13 of this work), and in consequence, considerably more time is necessary to plasmolyse a cell

with sugar solution than with potassium nitrate solution[1]. This is true for other kinds of cells as well as for guard cells. A strong solution can operate more rapidly than a weaker one, because the extracted water mixes with the agent, and forms a zone of diluted solution, which is in immediate contact with the film. If the solution employed is almost isosmotic with the cell sap, the liquid in the zone of dilution ceases to draw water from the cell until more solute has diffused from the agent into the zone of dilution. (Cf. **29** p. 23) It is not surprising then, that STEINBERGER had to wait 15 minutes when the molarity of the sugar solution was 0·2[2]. Error will result in O_g value of tissues not containing starch, as well of those which do, when the readings are taken before an isosmotic solution of the agent could cause plasmolysis (cf. tables 10, 11, 12, and 13). The fact that the film of plasm, in the cells to which STEINBERGER refers, maintained a constant position from the 15th to the 60th minute, argues very much in favor of sugar as an agent, and shows that, within that time, the O_g remained constant, i. e. neither penetration of the agent, nor molecular changes within the cell (tending to vary O_g) could have occurred.

BÄCHER (**1** p. 46) found that the extraction of water from the cells causes an increase in the O_g. The rate of variation is so slight however, that this factor cannot influence the measurement of O_g, by the accepted method.

While preferring potassium nitrate, STEINBERGER was conscious of its disadvantages as an agent. According to her data, guard cells immersed in a potassium nitrate solution, showed incipient plasmolysis as follows: After 5 minutes in a 0·4 molar solution; after 15 minutes in a 0·6 molar solution, and after 60 minutes in a 1·0 molar solution. She concludes from the results that potassium nitrate entered the cell. She thinks enzymatic activities are induced, for starch disappeared simultaneously in the cells. She evidently tried to avoid the difficulties referred to in the 3rd and 4th considerations by reducing the time of

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[1] This factor becomes particularly effective when the section remains mounted on the slide, or when the liquid is not agitated during the period of immersion. Wiggins kept the section mounted on the slide (**33** p. 32).

[2] FITTING cautioned against the error of not allowing sufficient time for plasmolysis: "—die Versuche wurden zu früh abgebrochen—d. h. die Plasmolyse wurde zu einer Zeit beurteilt, wo sie in den Zuckerlösungen noch nicht ihren Höhepunkt erreicht hatte etc". (7 p. 606). To obtain an impression of the degree of this error, see table 13 of this work.

immersion. She made the measurements between 5 and 10 minutes after immersion. In the effort to avoid these difficulties she introduced two errors which she deemed inevitable (**23** p. 4).

Consideration VII: Concentrated solutions of the agent are inclined to cause the walls of delicate cells to cave in. (Cf. **14** p. 225 and **29** p. 63). This is due to the fact that water passes out through the wall faster than the solution can diffuse inward, so that the difference of pressure between the inside and outside is greater than the wall can support. This is apt to happen more readily when sugar is employed than when potassium nitrate is used. This difficulty is not encountered when the walls are fairly rigid and the concentrations of the sugar solutions are not very high. In my experiments with leaves, I never noted the walls of any of the tissues to cave in, while immersed in the sugar solutions.

Consideration VIII: An agent that penetrates the plasm, might cause it to swell (e. g. KCNS), with consequent error in the O_g values. O_g readings, obtained with an agent that penetrates the plasm, must be suspected, until it is proven that the plasm does not vary in volume during the period of immersion.

From these considerations it follows that, the most important thing to determine is the constancy or the variation of O_g, after the sections have been immersed in the agent for different periods of time. A variation of O_g might indicate, penetration of the solute, (with or without attending complications) or molecular changes induced in the cell without penetration of the agent.

METHODS

The proposed osmotic studies referred to the tissues of leaves. Logically these tissues were chosen as subject matter for testing the reliability of the plasmolysing agents.

The volumetric normal system (1 gram-molecule per 1000 cm^3 of solution) was employed.

Since many tissues had to be studied in relatively short periods of time, it was impossible to reduce the graduation differences in the solutions (in the greater number of the experiments) below 0·05 mol.

In testing the agents five different methods were used.

(1) "Simultaneous"[1] $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ measurements were made on the tissues. The osmotic coefficients (i) were calculated and compared. Any variation in the permeability of the plasm should be indicated by a corresponding variation in i.

(2) $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ were measured for the tissues after different periods of immersion, in various concentrations of the proper agents. In an ideal agent a given tissue should show the same O_g, after any period of immersion, which is sufficient to allow plasmolysis to set in.

(3) Variations in the degree of plasmolysis were studied in one and the same cell. Cells under observation were sometimes kept in the field of vision during the test, and at other times the sections were returned to the flasks containing the agents. The degree of plasmolysis was studied (a) by drawings, (b) by time records of the position of the film.

(4) After $O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$ were determined for a given tissue, a cell was plasmolysed in a sugar solution, that corresponded to its $O_g(C_{12}H_{22}O_{11})$, after which it was placed in a potassium nitrate solution, and variations in the degree of plasmolysis were then recorded.

(5) After the $O_g(C_{12}H_{22}O_{11})$ of a cell was determined, the section was immersed in a potassium nitrate solution. The $O_g(C_{12}H_{22}O_{11})$ value of the cell was then determined again, and compared with the original $O_g(C_{12}H_{22}O_{11})$.

In all of the tests leaves of *Hedera Helix* were employed. The vine from which they were taken was vigorous, grew in a protected place, in good garden soil. It had an abundance of large leaves, which made it comparatively easy to pick them in pairs. Both leaves were always of about the same size, shape and age. No leaves were chosen from the flowering branches.

Before entering upon the regular experiments preliminary tests were made (July 21-30, '24) to determine the most favorable time of immersion, and to obtain threshold values for the different tissues. It soon became clear that sections (from any tissue) could be left in sugar solutions from 20 to 60 minutes without any variation in the O_g. After a certain length of time of immersion in potassium nitrate however, O_g increased in all of the tissues. A more exact study of the most

[1] The time between the measurements of $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ was actually about 10 or 15 minutes.

favorable time of immersion was made later. The results of the preliminary tests are recorded in table 1. The "simultaneous" measurements of $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ were made within the limits of time recorded.

Table 1
Limits of Time for the Immersion of Sections

	Potassium Nitrate		Cane Sugar	
	O_g in Mol	Limits of Time in Min.	O_g in Mol	Limits of Time in Min.
Upper Epidermis	0·45	5—10	0·6	15—60
Lower Epidermis	0·45	5—10	0·6	15—60
Guard Cells	0·5	7—12	0·65	15—60
Palisade Cells	0·8	15—20	0·9	20—60
Spongy Parenchyma . . .	0·6	5—10	0·75	15—60
Conducting Parenchyma . .	0·8	10—15	0·9	20—60

FIRST METHOD

Comparing Simultaneous $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ Measurements

Experiment I_a (Aug. 25—Sept. 2 '24)

One of the two leaves chosen, was placed in a vase filled with water, after the petiole had been sliced open for a distance of one cm. along its length. (According to previous experience, this operation insured a good water supply to the tissues of the lamina. Cf. 9 p. 45) Every 24 hours the water in the vase changed, the petiole was sliced open for one more cm., and a fresh transverse section was made near the end of it. This leaf was kept in a moist chamber, which consisted of a bell jar, standing in a vessel that contained water. Half of the inner surface of the bell was lined with moist filter paper, which dipped into the water below. A hygrometer suspended inside of the bell constantly registered 100% relative humidity. The leaf in the moist chamber (check) was exposed to diffuse day light, coming from a large window on the North side of the room. The test leaf (suspended beside the moist chamber) was allowed to wither while exposed to the same light conditions as the check. In order to insure normal transpiration, it was

freely suspended above the table as follows: A short wide glass tube was suspended in a vertical position; the leaf was placed into it; the petiole projected beyond the lower end of the tube for the greater part of its length (no pressure was exerted on the petiole); the lamina was supported on the (round) upper edge of the tube. Excepting the relative humidity and the water supply, the influencing factors were about the same for both leaves.

The $O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$ for a given tissue were measured first for the test leaf and immediately afterward for the check. The order in which the measurements on the tissues were made, was the same as that in which they appear in the table. The measurements on a given tissue followed each other after intervals of 24 hours.

The results obtained are recorded in table 2. When plasmolysis occurred normally, and when the cell was quickly deplasmolysed upon being placed in pure water, the O_g obtained was accepted; but when deplasmolysis failed to set in or occurred very slowly, the tissue was considered dead and a (+) was placed in the table to indicate its condition. A (—) indicates that no measurement was made.

The first effects of drying were noted in the petiole, which began to shrivel near its point of attachment to the lamina[1]. On the fifth day the spongy parenchyma began to show an abnormally great increase in O_g. On the sixth day the results became doubtful: Some of the cells that were apparently plasmolysed, failed to deplasmolyse quickly in water, while others deplasmolysed normally. On the seventh day the majority of the cells of the spongy parenchyma were dead. The mesophyll had shrunken considerably on this day, and the epidermis had caved in somewhat, which made it difficult to make satisfactory sections (the stiff fibro-vascular bundles formed ridges). The palisades behaved about the same as the spongy parenchyma. The conducting parenchyma died on the sixth day. The guard cells and the epidermal tissues were still alive on the eighth day. All of the tissues of the check were alive at the termination of the experiment.

The pressure in atmospheres, equivalent to the $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$, have not been added to the table, because there is no reliable table of equivalents on hand for potassium nitrate.

One should be able to obtain an impression of the behavior of the two agents in the study of the various tissues, by calculating the

[1] This agrees with the experience of HAYOZ (9 p. 31).

Table

Exp. 1 a Simultaneous $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$

		$O_g(KNO_3)$					
		Day of Test					
		1	2	3	4	5	6
Test	Upper Epidermis	0·45	0·45	0·45	0·45	0·5	0·5
	Palisades	0·8	0·85	0·9	0·95	1·05	1·2
	Conducting Parenchym	0·85	1·0	1·1	1·15	1·3	+
	Spongy Parenchym	0·65	0·75	0·8	0·95	1·1	1·3
	Guard Cells	0·45	0·5	0·5	0·55	0·55	0·65
	Lower Epidermis	0·45	0·45	0·45	0·5	0·55	0·6
Check	Upper Epidermis	0·45	0·45	0·45	0·45	0·5	0·55
	Palisades	0·75	0·75	0·8	0·8	0·85	0·9
	Conducting Parenchyma	0·85	0·85	0·85	0·9	0·95	0·95
	Spongy Parenchyma	0·65	0·7	0·7	0·75	0·75	0·85
	Guard Cells	0·45	0·45	0·45	0·6	0·5	0·6
	Lower Epidermis	0·45	0·5	0·5	0·55	0·55	0·6

osmotic coefficients (2 Vol. I p. 28). This is the basis of the method employed by LEPESCHKIN (16), TRÖNDLE (25) and others, to determine the degree of penetration of a solute.

The osmotic coefficient (i) is given by the formula:

$$i = \frac{O_g(C_{12}H_{22}O_{11})}{O_g(KNO_3)}$$

The results of the calculations of i from the simultaneous O_g measurements (recorded in table 2) are given in table 3.

The variations of i noted in the table, might be the result of variations in $O_g(KNO_3)$, variations in $O_g(C_{12}H_{22}O_{11})$ or variations in both O_g values. It is impossible to determine variations in O_g and judge the degree of permeability, unless the error resulting from the methods be known.

The approximation of O_g may evidently differ from the absolute value by $\pm$ 0·025 mol. The per cent error is greater for low O_g values than for high O_g values. The possible per cent error in O_g from this source, at the various concentrations, is as follows:

Concentration in mol .	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9	1·0
% Error in O_g . . .	25	12·5	8·2	6·2	5	4	3·5	3	2·8	2·5

2

Values of a Drying Leaf and Check

$O_8(KNO_3)$			$O_8(C_{12}H_{22}O_{11})$								
Day of Test			Day of Test								
7	8	9	1	2	3	4	5	6	7	8	9
0·55	0·55	—	0·6	0·55	0·6	0·6	0·65	0·6	0·7	—	—
+	+	+	0·9	0·95	1·05	1·1	1·2	1·4	+	+	+
+	+	+	0·95	1·1	1·2	1·25	1·4	+	+	+	+
+	+	+	0·75	0.85	0·95	1·05	1·2	1·4	+	+	+
0·9	—	—	0·6	0·65	0·7	0·75	0·75	0·8	1·4	—	—
—	—	—	0·6	0·6	0·65	0·7	0·7	0·75	—	—	—
0·55	0·55	0·55	0·55	0·55	0·6	0·55	0·6	0·7	0·65	0·65	—
—	—	—	0·85	0·9	0·85	0·85	0·9	0·95	—	—	—
—	—	—	0·95	0·9	0·95	0·95	1·0	1·0	—	—	—
—	—	—	0·8	0·8	0·8	0·8	0·8	0·85	—	—	—
0·7	—	—	0·65	0·6	0·65	0·75	0·65	0·75	0·85	—	—
0·65	—	—	0·65	0·65	0·65	0·7	0·7	0·75	0·8	—	—

Table 3

Osmotic Coefficients obtained from Table 2

$i = \frac{O_8(C_{12}H_{22}O_{11})}{O_8(KNO_3)}$		Day of Test							
		1	2	3	4	5	6	7	8
Upper Epidermis	Test	1·33	1·22	1·33	1·33	1·33	1·2	1·27	—
	Check	1·22	1·22	1·33	1·22	1·2	1·27	1·18	1·18
Palisades	Test	1·125	1·12	1·17	1·16	1·14	1·17	—	—
	Check	1·13	1·2	1·06	1·06	1·06	1·05	—	—
Conducting Parenchyma	Test	1·11	1·1	1·09	1·08	1·07	—	—	—
	Check	1·11	1·06	1·11	1·05	1·05	1·05	—	—
Spongy Parenchyma	Test	1·15	1·13	1·19	1·11	1·09	1·08	—	—
	Check	1·23	1·14	1·14	1·07	1·07	1·0	—	—
Guard Cells	Test	1·33	1·3	1·4	1·36	1·36	1·23	1·56	—
	Check	1·44	1·44	1·44	1·25	1·3	1·25	1·21	—
Lower Epidermis	Test	1·33	1·33	1·44	1·4	1·27	1·25	—	—
	Check	1·44	1·3	1·3	1·27	1·27	1·25	1·23	—

The error in O_g evidently affects the value of **i**. Consider **i** to be constantly equal to 1·0 for all of the various concentrations, so that an estimate of the per cent error can be made for **i**, when the maximum errors are made in $O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$, and that these are such as to produce maximum error in **i**. The estimate will be greater than the actual error, since **i** is always greater than 1·0. At the concentration 0·1, 0·075 might be the correct value of $O_g(KNO_3)$, and 0·125 might be the correct value of $O_g(C_{12}H_{22}O_{11})$. The actual value of **i** would then be 1·66 instead of 1·0, and the error would be (1·66—1·0) or 0·66. The maximum per cent error possible, under the conditions, at the various concentrations, would be[1]:

Concentration in mol .	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9	1·0
% Error in **i** . . .	66	28	18	13	10	8·6	7·3	6·4	5·7	5

In table 2 there are no values for O_g below 0·45 mol, and hence the error in **i** cannot be greater than 12%. Furthermore, the value of **i** as given in table 3 is greater than 1·0 in all cases save one; this fact further reduces the actual per cent error. By way of illustration, consider **i** for the upper epidermis (low O_g) of the test leaf on the first day; its value is 1·33 resulting from $O_g(KNO_3) = 0{\cdot}45$, and $O_g(C_{12}H_{22}O_{11}) = 0{\cdot}6$. Suppose the maximum error were made, i. e. the absolute value of $O_g(KNO_3)$ were 0·425 and the absolute $O_g(C_{12}H_{22}O_{11})$ were 0·625, then **i** would be 1·47, instead of 1·33, which makes a difference of 9·5% from the value given. (According to the table it is less than 10%). Consider the value of **i** for the conducting parenchyma on the first day (high O_g). The error must be less than 6·4%, according to the table (the $O_g(C_{12}H_{22}O_{11})$ being 0·95, and $O_g(KNO_3)$ 0·85, and **i** 1·1); Suppose the true value of $O_g C_{12}H_{22}O_{11})$ were 0·975 and $O_g(KNO_3)$ 0·825, then **i** would be 1·18, so that error would be 5·1%.

The initial values of **i** (3rd column of table 3) are the same for the test and check, for a given tissue, in only one case (conducting parenchyma); the difference however, is always within the limits of error resulting from the methods. To illustrate, consider the lower epidermis (O_g being low, the possible error is high), the possible error in 1·44 is $\pm$ 0·2 and the probable error is not more than $\pm$ 0·1, so that instead

[1] The actual error cannot be calculated, since it is not known how much **i** differs from 1·0.

of 1·44 the value might be 1·24 or 1·64, but probably not less than 1·34 nor more than 1·54.

The difference between the initial values of i in different tissues for the check (or the test) are beyond the limits of possible error due to the methods. To illustrate, consider the conducting parenchyma and the lower epidermis. The possible error in 1·11 is $\pm$ 0·07 and the probable error is not more than $\pm$ 0·035 so that instead of 1·11 the value might be 1·04 or 1·18 and probably is not less than 1·075 nor more than 1·145. The lower epidermis is probably not less than 1·34 nor more than 1·54. Evidently the i values of the different tissues of a fresh leaf need not be the same (possible reasons will be given later).

The epidermal tissues and the mesophyll tissues (not the guard cells) show a tendency to decrease in i, as drying proceeds. From the equation,

$$i = \frac{O_g(C_{12}H_{22}O_{11})}{O_g(KNO_3)}$$

it is clear that i may decrease: Ist because $O_g(C_{12}H_{22}O_{11})$ is less than it should be normally after the tissue suffered from drought; 2nd because $O_g(KNO_3)$ is greater than it should be normally; 3rd because $O_g(C_{12}H_{22}O_{11})$ is greater, and $O_g(KNO_3)$ is less than it should be normally. The results of previous investigators (as well as my own, which will follow) indicate that the plasm is not permeable for sugar, so that in accepting this method of judging the variation in permeability by the variation of i, any change in i must be referred to the agent which is compared with the sugar. Viewed in this light, i has decreased as drying proceeded, because the plasm has become more permeable for potassium nitrate.

In this connection, it is worthy of notice, that the guard cells of the test leaf alone show an (irregular) increase from the initial to the final value. If exception be made of the reading on the seventh day, the variations from the initial 1·33 value are within the possible error due to the methods. The tendency to increase is made more significant however, by the fact that the check shows an evident decrease in i values.

There are proofs on hand, which will be published later, that the guard cells manifest an evident diurnal periodicity. In general they are very sensitive to the influence of various exterior factors, and can respond by a considerable variation in O_g[1]. It will be shown later (see

[1] Compare the findings of Iljin, Steinberger, Wiggins and Ursprung and Blum 29 p. 18.

table 4) that a variation in O_g (without a variation in permeability) can cause a variation in **i**. If the O_g varies considerably in the guard cells, it evidently follows that the **i** values are not very reliable as indicators of the permeability relations, and that fluctuations noted in the **i** values must be expected. (Compare FITTING 8 p. 169).

The most striking thing about the entire table is the fact that all of the values for **i** are considerably below the value which FITTING puts down as the best figure for potassium nitrate i. e., $\mathbf{i} = 1{\cdot}64$ (7, p. 609). Did the potassium nitrate penetrate in all of the tissues, in spite of the precaution to operate as quickly as possible? Did the O_g increase before plasmolysis could set in? In that case evidently the value, that was accepted as being isosmotic with a given cane sugar solution, was actually hypertonic. Before drawing any conclusion the following considerations must be borne in mind.

(1) The entire method of determining the variations of permeability by the variations of the isosmotic coefficient depends upon the theory of VAN'T HOFF and the law of ARRHENIUS, which hold only at the limiting values of infinitely dilute solutions. If the theory of VAN'T HOFF had a more secure physical foundation, or if we had reliable data obtained by direct pressure measurements for potassium nitrate solutions, it might be possible to determine a correction factor (similar to the one applied to the law of BOYLE for pressures beyond 10 atmospheres), when dealing with values greater than the limiting value. It is evident from this, that the values in question can only be compared with those obtained by FITTING, when one operates with solutions of about the same concentrations as he employed. Unfortunately the nature of this work does not permit a reduction in the concentrations of the solutions, and there is no correction factor on hand that might be applied.

(2) FITTING naturally employed tissues which manifested low O_g values, namely between 0·09 and 0·12 mol potassium nitrate and 0·16 and 0·2 mol cane sugar. The O_g values for the tissues which were best suited for my work ranged from 0·45 to 1·3 mol potassium nitrate and from 0·6 to 1·5 mol cane sugar.

(3) It is possible, even very probable, that the **i** value decreases automatically with the increase in concentration, so that isosmotic values equivalent to 10 atmospheres, for example, would give a lower value than isosmotic values for 5 atmospheres. This appears from table 4, in which the osmotic values and the corresponding **i** values are given for $CaCl_2$, NaCl, and KNO_3 at concentrations equivalent to 4, 5, 10, 20,

30, 38·8, and 40 atmospheres. The values for i decrease as the values for the atmospheres, and their equivalent molarities, increase.

Table 4

Value of i at Different Concentrations

Equivalent Atmospheres	Concentration of the Salt in Mol				$i = \frac{\text{Mol } C_{12}H_{22}O_{11}}{\text{Mol Salt}}$		
	$CaCl_2$	NaCl	KNO_3	$C_{12}H_{22}O_{11}$	$CaCl_2$	NaCl	KNO_3
4	—	—	0·10	0·16	—	—	1·6
5	—	0·107	0·125	0·20	—	1·87	1·6
10	0·12	0·22	0·255	0·375	3·01	1·7	1·5
20	0·33	0·44	0·5	0·675	2·05	1·54	1·35
30	0·47	0·66	0·75	0·91	1·94	1·38	1·21
38·8	0·6	0·87	1·105	1·105	1·84	1·27	1·0
40	0·605	0·88	—	1·11	1·81	1·26	—

The values in the tables were obtained as follows: Graphs were drawn on a large scale, expressing the relations between the concentrations of the solutions and the equivalent pressures in atmospheres. From these graphs the given values were read. The data for the $CaCl_2$ graph were taken from the calculations of WIGGINS (33 p. 31) who employed the formula and the tables of JONES (based on the depression of the freezing point, and concentration of the solution). The data for the NaCl and KNO_3 are taken from STEINBERGER (23 p. 26), who obtained them by straight line interpolation of the results of RENNER's calculations (based on the depression of the freezing point, and the concentration of the solution). The data for the sugar curve were taken from the tables of URSPRUNG and BLUM (28 p. 533) who interpolated the results of MORSE and BERKELEY and HARTLEY. (Direct measurements).

If the data thus obtained can be applied to tissues and cells, it is evident that, at equal pressures, the corresponding concentrations should be isosmotic, and the calculated i values should be reliable. For $CaCl_2$ there were no data on hand to calculate i at 4 and 5 atmospheres. From 10 to 40 atmospheres i varies from 3·01 to 1·81 for $CaCl_2$. For NaCl there were no data on hand below 5 atmospheres. From 5 atmospheres to 40 atmospheres i varies from 1·87 to 1·26 for NaCl.

For KNO_3 **i** varies from 1·6 to 1·0, in going from 4 atmospheres to 38·8 atmospheres.

Thus it follows, that quite independent of permeability relations, the **i** value should decrease with increasing O_g values.

Experiment I_b

This experiment was in the main, a repetition of the experiment I_a (table 2). Instead of suspending a leaf to dry, two were placed on the table to dry, one having the upper epidermis turned upward. The results were not much different from those furnished by experiment I_a, and nothing new could be learned from them regarding the relative values of cane sugar and potassium nitrate as a plasmolysing agent, and the variation of the osmotic coefficient **i**, so that tables are not given here.

These two experiments suggested the following improvements in the methods:

(1) Give closer attention to each tissue, and make simultaneous measurements of $O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$ on only one tissue at a time, and repeat the tests several times to guard against errors due to faulty observations.

(2) Increase the degree of exactness of the work by reducing the possible error in O_g and **i**, by making the graduation differences in the solutions 0·025 mol, instead of 0·05 mol.

(3) Preclude possible error of judgment, or error of observation, by calculating the $O_g(C_{12}H_{22}O_{11})$ from the **i** value given by FITTING (1·64) and the measured $O_g(KNO_3)$; furthermore by calculating the $O_g(KNO_3)$ from the same **i** value (1·64) and the measured $O_g(C_{12}H_{22}O_{11})$; and finally by observing the degree of plasmolysis obtained by immersing test sections in the molarities equal to the calculated $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ values, observing the degree of plasmolysis produced.

These suggestions were carried out, and the results were assembled, under the head of Experiment I_c, in table 5.

Experiment I_c

In reading table 5 it must be remembered that the various tissues were measured at different times as follows: The upper epidermis Sept. (10—19); the palisades Nov. (24—29); the conducting parenchyma Nov. (19—24); the spongy parenchyma Dec. (1—7); the lower epidermis and the guard cells Dec. (9—17). The Experiments I_a and I_b were

Table 5

Simultaneous $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ Values of Drying Leaves, Exp. 1c and Derived i Values

	Day of Test	Upper Epidermis	Palisades	Conducting Parenchyma	Spongy Parenchyma	Guard Cells	Lower Epidermis
$O_g(KNO_3)$	1	0·525	0·9	0·9	0·675	0·65	0·625
	2	0·5	0·975	0·975	0·75	0·7	0·65
	3	0·5	1·075	1·05	0·825	0·7	0·625
	4	0·525	1·25	1·175	0·95	0·7	0·625
	5	0·525	1·5	+	1·1	0·7	0·625
	6	0·375	+		1·3	0·75	0·625
	7	0·35			+	0·75	0·6
	8	0·65				0·775	0·55
	9	0·5				0·8	0·525
	10	+				+	+
$O_g(C_{12}H_{22}O_{11})$	1	0·675	1·0	1·05	0·775	0·775	0·75
	2	0·65	1·1	1·125	0·85	0·825	0·75
	3	0·65	1·2	1·125	0·95	0·825	0·75
	4	0·675	1·35	1·35	1·05	0·825	0·75
	5	0·675	1·6	+	1·2	0·825	0·75
	6	0·5	+		1·3	0·9	0·75
	7	0·4			+	0·9	0·7
	8	0·85				0·925	0·625
	9	0·65				1·0	0·625
	10	+				+	+
$i = \frac{O_g(C_{12}H_{22}O_{11})}{O_g(KNO_3)}$	1	1·285 ± 0·065	1·11 ± 0·03	1·165 ± 0·03	1·15 ± 0·04	1·19 ± 0·035	1·2 ± 0·045
	2	1·297 ± 0·06	1·13 ± 0·025	1·165 ± 0·02	1·13 ± 0·03	1·18 ± 0·03	1·2 ± 0·045
	3	1·297 ± 0·065	1·12 ± 0·02	1·075 ± 0·02	1·15 ± 0·025	1·18 ± 0·03	1·15 ± 0·04
	4	1·275 ± 0·065	1·08 ± 0·02	1·14 ± 0·02	1·105 ± 0·02	1·18 ± 0·03	1·2 ± 0·045
	5	1·275 ± 0·065	1.07 ± 0·01	+	1·09 ± 0·015	1·18 ± 0·03	1·2 ± 0·045
	6	1·33 ± 0·07	+		1·0 ± 0·01	1·2 ± 0·03	1·2 ± 0·045
	7	1·14 ± 0·08			+	1·2 ± 0·03	1·17 ± 0·045
	8	1·3 ± 0·05				1·25 ± 0·03	1·135 ± 0·05
	9	1·297 ± 0·065				1·25 ± 0·03	1·19 ± 0·05

performed in the Summer, and the tests described in this section were made late in the Fall of 1924.

The $O_g(C_{12}H_{22}O_{11})$, calculated from FITTING's value $i = 1{\cdot}64$ and the measured $O_g(KNO_3)$, was always found to be considerably too high for all of the tissues. Sections, when placed in the equivalent sugar

solutions, always showed the cells of all of the tissues to be excessively plasmolysed. The $O_g(KNO_3)$ calculated from the same value of **i** und the measured $O_g(C_{12}H_{22}O_{11})$ was always found to be too low, so that the cells of all of the tissues failed to plasmolyse in the equivalent potassium nitrate solution. After many checks were made in the manner suggested above, there could be no doubt but that the difference between the calculated value of **i**, and the value given by FITTING, could not be due to errors of observation or of method.

The **i** value obtained from the measured $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ have been included in table 5. The maximum error possible in the calculation of **i**, resulting from the methods, have been appended to each value. The probable error is hardly greater than one half of this value, since the errors in O_g would have to be extreme in both measurements, which could hardly have occurred without being remarked in the percentage count and the degree of plasmolysis.

The **i** values for the upper epidermis varied from an initial 1·285 to 1·33 on the 6th day and then fell to 1·14 on the 7th day, after which it suddenly rose to 1·3 on the 8th day and then fell to 1·297. The temporary rise on the 6th day may have resulted from an error (the difference between the **i** values for the 5th and 6th day is within the limits of the probable error). This view is supported by the fact that the **i** value is lower on the 7th day. The decrease of O_g would probably have continued on the 8th day if disturbing factors (not within my control) had not entered. The temperature in the laboratory dropped on that day from the usual 20° to 11° C., with the result that O_g increased considerably. An increase in O_g should cause **i** to decrease (cf. table 4), but it is possible that the lower temperature decreased the permeability for potassium nitrate; in fact the readings on the 9th day favor this view: Although $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ dropped again as the temperature rose, nevertheless **i** decreased, whereas, other things remaining equal, it should have increased with the decrease of O_g. — The initial O_g values are higher than they were in Summer [0·45 (KNO_3) and 0·6 $(C_{12}H_{22}O_{11})$] but the initial **i** value is about the same now as in Summer. In Summer the initial **i** value was 1·33 for the test and 1·22 for the check (cf. table 3), and in Fall it was 1·285 (cf. table 5). From this it would appear that low temperature increases the O_g, and also increases **i**, i. e. it decreases the permeability in this tissue[1]. Drought

[1] These three indications support the view of FITTING, that cells are hardly permeable for KNO_3 in Winter. (Cf. 7 p. 608).

increases O_g but decreases **i** i. e. the permeability for KNO_3 seems to increase with the lack of water in this tissue.

The lower epidermis shows an initial **i** value of 1·2. This is lower than the value recorded in Summer (1·33 or 1·44 cf. table 3). It must be remembered that the O_g value is higher than in Summer, which might explain the lower initial **i** value. The lowest **i** value is reached on the 8th day (1·135), but when we consider the probable error (0·03) the variation from the initial 1·2 is slight.

The upper epidermis and the lower epidermis show an unmistakable tendency to decrease their O_g values. In the lower epidermis it is more gradual than in the upper epidermis, where it occurred suddenly on the 6th day. This decrease seems to be exceptional, since the other tissues fail to respond in the same way, and since it was not noted in the epidermal tissues in experiment I_a (table 2)[1]; still it is noted here in two different leaves at different times (i. e. upper epidermis on one leaf on Nov. 10 and lower epidermis on another Dec. 9). The fact that in two other leaves, both upper and lower epidermis showed a marked and more or less regular decrease, makes this phenomenon even more interesting: In experiment I_b the two epidermal tissues of both drying leaves decreased from an initial $O_g(KNO_3)$ (0·55) mol, and $O_g(C_{12}H_{22}O_{11})$ (0·7) mol, to $O_g(KNO_3)$ (0·15) mol, and $O_g(C_{12}H_{22}O_{11})$ (0·3) mol, on the 9th day.

The palisades show a slight decrease in **i** values. A greater decrease might have been expected, since the O_g values increase considerably. In experiment I_b the **i** value for this tissue failed to decrease in both drying leaves, even though the O_g values increased considerably. In experiment I_a (table 3) there was a slight increase in the **i** values, in spite of the increase in the O_g values. These results suggest that the palisades are less permeable for KNO_3 when withering than when fresh.

The slight decrease of the **i** values for the conducting parenchyma is not as great as might have been expected for the corresponding increase in O_g. The decrease was slightly greater for the withering leaves of experiment I_b, and also in experiment I_a (table 3). In general this tissue behaved like the palisades.

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[1] BAECHER found that O_g increases when the relative humidity of the air is low and when the soil is dry (**1**, p. 54). URSPRUNG and BLUM found a similar result (**30**, p. 136).

The **i** values for the spongy parenchyma decrease more regularly and more considerably than the **i** values for the palisades and the conducting parenchyma. In experiment I_a and experiment I_b, the decrease in **i** was slight.

The guard cells show an evident increase in the **i** values, in spite of the increase in the O_g values. The same is true for the **i** values calculated in experiment I_b and experiment I_a (table 3). It is worthy of note that these cells reacted differently than the other tissues in every one of the experiments. Furthermore the initial **i** values for these cells is lower (1·19), late in Fall, than in Summer (1·33). If increased permeability is indicated by a decrease in **i**, the cold seems to make these cells more permeable for KNO_3 while drought seems to make them less permeable. This is just the opposite from what seems to be the case in the epidermal tissues. The manner of the variation of the permeability seems to be the same as for the tissues of the mesophyll under the influence of drought and cold, but in a more marked degree in the guard cells than in the mesophyll tissues. The data on hand are not sufficient to allow a definite and conclusive statement to this effect. The indication seems clear enough to warrant further experiments, in which attention is confined to the influence of these factors on the permeability relations in the guard cells, epidermal cells, and the tissues of the mesophyll.

In studying these variations of the **i** values, it must be remembered that the time of immersion in the KNO_3 was reduced as nearly as possible to the minimum of time necessary to plasmolyse the tissue.

While this method of studying the permeability of the plasm may be instructive it does not inspire much confidence. It can hardly serve when quantitative results are sought.

Experiment II_a (September, 15—23, 1924)

Two leaves were chosen in the usual manner. Instead of allowing them to dry in diffuse daylight one was left to dry suspended in a dark chamber and the other in the light of a 2500 C. P. tungsten lamp.

The dark chamber was in a dark room, where all measurements were made in the light of a 16 C. P. filament lamp. The arrangement was such that the leaf in the dark room was not exposed to the light of the weak lamp more than 15 minutes per day (the time necessary to make the sections). The room in which the other leaf was exposed to the light, communicated with the dark room. An asbestos screen,

having a circular hole in it, was placed in front of the 2500 C. P. lamp, so that it cut off all unnecessary radiation in the direction of the leaf. Before reaching the leaf, the beam of light was passed through a cooling tank of large capacity, having two plane-parallel glass faces. By actual test, the temperature remained constant for more than one hour, at the point where the leaf was exposed to the light. Throughout the experiment the temperature did not vary more than 1° above or below 20° C. at this point. The air of the to rooms was circulated from time to time, by means of an electric fan (precautions being taken that no air currents struck the leaves under test). The temperature and humidity conditions were almost the same for both leaves throughout the experiment. The illuminating device was kept in operation continuously during the experiment, i. e. day and night for 8 days.

The results of this experiment are given in table 6.

Although the two leaves were fresh in appearance, turgescent and olive-green in color, all their tissues showed an abnormal reaction from the outset of the experiment.

The upper epidermis showed an initial O_g, 0·45 (KNO_3) and 0·6 mol ($C_{12}H_{22}O_{11}$). Just a week before, three leaves from the same vine, of about the same age and size, showed higher values [0·55 (KNO_3) and 0·7 ($C_{12}H_{22}O_{11}$)]. Two weeks before (see table 2) the same values were found (0·45 and 0·6). About two months later (table 5) 0·525 $O_g(KNO_3)$ and 0·675 $O_g(C_{12}H_{22}O_{11})$ were found. The usual tendency to drop in O_g value is again noted in both withering leaves but already on the 6th day, O_g stood higher than the initial value. On the 8th day the tissue was dead. (It remained alive more than 8 days in all previous tests).

The lower epidermis of the leaf in the dark failed to show the usual drop in O_g but that of the one in the light showed it (as the upper epidermis) on the second day already (unusual). On the 6th day both leaves showed an O_g value that was higher than the initial value, and on the 8th day the tissue of the leaf in the dark was dead, but not that of the leaf exposed to the light.

The tissues of the mesophyll began with abnormally high O_g values, especially the palisades, and soon died after showing considerable increase in O_g.

The guard cells, which ordinarily are very resistant and live a long time in drying leaves, began with normal O_g values, which increased rapidly (more in the light than in the dark) and died already on the 6th day.

Table

Exp. IIa Simultaneous $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ Values of a

		$O_g(KNO_3)$				
		Day of the				
		1	2	3	4	5
Leaf in the Light	Upper Epidermis	0·45	0·4	0·4	0·4	0·45
	Palisades	1·0	1·2	1·3	+	
	Conducting Parenchyma	0·9	1·0	1·05	+	
	Spongy Parenchyma	0·6	0·8	0·85	—	1·0
	Guard Cells	0·45	0·65	0·7	—	0·95
	Lower Epidermis	0·45	0·85	0·3	0·4	0·45
Leaf in the Dark	Upper Epidermis	0·45	0·4	0·4	0·45	0·45
	Palisades	1·1	1·2	1·3	+	
	Conducting Parenchyma	0·9	0·9	0·95	+	
	Spongy Parenchyma	0·6	0·75	0·75	—	0·8
	Guard Cells	0·45	0·5	0·6	—	0·8
	Lower Epidermis	0·45	0·45	0·5	0·55	0·6

The anomalies manifested by these leaves are interesting but make them unfit for drawing conclusions regarding permeability relations. The experiment was repeated, and is described as experiment II_b

Experiment II_b

This is a repetition of the previous experiment. The leaves still manifest certain anomalies (see table 7), in spite of the healthy appearance of the leaves. The results are recorded in table 7 together with the deduced **i** values.

The O_g values of the epidermal tissues showed an unusual variation. Furthermore the tissues died much earlier than usual. (They were dead on the 6th day). The steady and considerable rise of **i** for this tissue of the leaf in the dark is remarkable. A phenomenon, which was noticed in the previous, as well as in the present experiment, may have some relation with the increase in **i**: About two days after the leaf was in the dark, it became difficult to distinguish the film as it receded from the cell wall, in those sections which were immersed in sugar solution. This was not true for the sections immersed in potassium

6

Leaf drying in the Light and Another drying in the Dark

$O_g(KNO_3)$			$O_g(C_{12}H_{22}O_{11})$							
Test			Day of the Test							
6	7	8	1	2	3	4	5	6	7	8
0·5	0·6	+	0·6	0·55	0·55	0·6	0·65	0·65	0·7	+
			1·15	1·3	1·4	+				
			1·0	—	1·2	+				
+			0·75	—	0·95	—	1·2	+		
+			0·6	0·75	0·85	—	1·1	+		
0·5	0·5	0·5	0·65	0·55	0·5	0·6	0·65	0·65	0·7	0·7
0·6	0·6	+	0·6	0·55	0·55	0·65	0·6	0·7	0·7	+
			1·25	1·35	1·45	+				
			1·0	—	1·15	+				
+			0·75	—	0·8	—	0·9	+		
+			0·6	0·65	0·75	—	0·95	+		
0·65	0·65	+	0·65	0·6	0·7	0·75	0·8	0·8	0·8	+

nitrate solution. At the same time no such difficulty was experienced with sections taken from the leaf in the light. The difficulty increased with the continued exposure of the leaf in the dark. When studying sections from the leaf in the dark room, for some reason or other, there seemed to be little difference between the indices of refraction of the sugar solution and the contents of the cell. It is possible that 50% of the cells were plasmolysed but that plasmolysis could not be observed, in spite of repeated trials, in concentrations less than those given in the tables for sugar solutions[1].

The upper epidermis of the leaf drying in the dark showed the usual tendency to decrease in O_g (more in KNO_3 than in $C_{12}H_{22}O_{11}$, which would indicate that the plasm is less permeable for KNO_3 or has become permeable for sugar). Finally the $O_g(C_{12}H_{22}O_{11})$ increased 0·1 mol,

[1] The experience of most investigators seems to be that the plasm is impermeable for sugar. In very careful tests to be spoken of later, the plasm always manifested itself impermeable for sugar, which makes the experience referred to in this tissue most strange. More careful study of the phenomenon at some future time will probably reveal some interesting relations. (Compare FITTING 7, p. 608.)

Exp. IIb Table

Simultaneous $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ Values of a Leaf drying in

		$O_g(KNO_3)$					$O_g(C_{12}H_{22}O_{11})$				
		Day of the Test					Day of the Test				
		1	2	3	4	5	1	2	3	4	5
Leaf in the Light	Upper Epidermis . . .	0·4	0·4	0·4	0·4	0·45	0·55	0·55	0·6	0·7	—
	Palisades	1·0	1·15	1·3	1·4	+	1·15	1·25	1·3	1·4	+
	Conducting Parenchyma .	0·65	0·8	0·95	1·15	+	0·8	0·9	1·1	1·25	+
	Spongy Parenchyma . .	0·55	0·6	0·7	0·8	+	0·7	0·8	0·85	0·95	+
	Guard Cells	0·45	0·65	0·75	0·8	0·95	0·6	0·85	0·9	1·0	1·1
	Lower Epidermis . . .	0·4	0·45	0·5	0·55	0·9	0·55	0·6	0·65	0·7	—
Leaf in the Dark	Upper Epidermis . . .	0·45	0·4	0·3	0.3	0·45	0·6	0·55	0·6	0·7	—
	Palisades	1·0	1·05	1·05	1·15	+	1·15	1·15	1·15	1·2	+
	Conducting Parenchyma .	0·65	0·7	0·75	0·85	+	0·8	0·85	0·9	1·0	+
	Spongy Parenchyma . .	0·55	0·55	0·55	+		0·7	0·7	0·7	+	
	Guard Cells	0·45	0·4	0·4	0·45	+	0·6	0·55	0·6	0·65	+
	Lower Epidermis . . .	0·4	0·4	0·4	0·4	0·4	0·55	0·55	0·6	0·8	+

while $O_g(KNO_3)$ had reached the initial O_g. Either the plasm is less permeable for KNO_3 or more permeable for sugar. The **i** value varied from 1·33 to 2·3, which leads to the same conclusion. This tissue for the leaf, drying in the light, showed only an increase of 0·05 mol in $O_g(KNO_3)$ but $O_g(C_{12}H_{21}O_{11})$ increased 0·15 mol. Accordingly **i** increased from 1·385 to 1·75. Apparently the plasm became less permeable for potassium nitrate or became permeable for sugar. The decrease in $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ which was noted several times in withering leaves, and which is noted in this experiment for the leaf in the dark, did not set in for the leaf drying in the light.

In the lower epidermis no decrease in $O_g(KNO_3)$ or $O_g(C_{12}H_{22}O_{11})$ is noted in either leaf. For the leaf in the dark $O_g(KNO_3)$ remained constant but $O_g(C_{12}H_{22}O_{11})$ increased; as a result **i** increased from 1·385 to 2·0. As in the upper epidermis, the plasm either became less permeable for KNO_3 or became permeable for sugar. For this tissue of the leaf exposed to the light, $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ increased 0·05 mol on the second, third and fourth days[1]. Since 0·05 mol KNO_3

[1] The reading for $O_g(C_{12}H_{22}O_{11})$ was missed on the 5th day for unavoidable reasons.

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the Light and Another drying in the Dark and the Deduced Values of i

$$i = \frac{O_g(C_{12}H_{22}O_{11})}{O_g(KNO_3)}$$

Day of the Test				
1	2	3	4	5
1·385 ± 0·15	1·385 ± 0·15	1·5 ± 0·15	1·75 ± 0·18	—
1·15 ± 0·055	1·085 ± 0·045	1·0 ± 0·035	1·0 ± 0·035	+
1·23 ± 0·08	1·12 ± 0·07	1·16 ± 0·06	1·09 ± 0·045	+
1·27 ± 0·11	1·33 ± 0·11	1·21 ± 0·08	1·19 ± 0·07	+
1·33 ± 0·15	1·31 ± 0·09	1·21 ± 0·08	1·25 ± 0·07	1·16 ± 0·06
1·385 ± 0·15	1·33 ± 0·15	1·3 ± 0·12	1·27 ± 0·11	—
1·33 ± 0·15	1·385 ± 0·16	2·0 ± 0·25	2·3 ± 0·3	—
1·15 ± 0·06	1·09 ± 0·05	1·09 ± 0·05	1·04 ± 0·04	+
1·23 ± 0·09	1·22 ± 0·08	1·2 ± 0·08	1·18 ± 0·07	+
1·27 ± 0·11	1·27 ± 0·11	1·27 ± 0·11	+	
1·33 ± 0·15	1·385 ± 0·15	1·5 ± 0·15	1·44 ± 0·15	+
1·385 ± 0·15	1·385 ± 0·15	1·5 ± 0·15	2·0 ± 0·2	—

represents a higher osmotic value than 0·05 mol $C_{12}H_{22}O_{11}$ it is evident that the permeability of the plasm for KNO_3 has increased or that its permeability for sugar has decreased. This conclusion is legitimate, even though the decrease in i is not great.

The initial O_g of the palisades is high, just as in the previous experiment (compare the values in table 7 with those given in tables 2, 5, and 6). As drying proceeds, O_g increases for both leaves. For the leaf in the light O_g increased 0·4 mol (KNO_3) and only 0·25 mol ($C_{12}H_{22}O_{11}$); i decreased, once because O_g increased, and then because $O_g(KNO_3)$ increased more than $O_g(C_{12}H_{22}O_{11})$. The plasm has become more permeable for potassium nitrate or less permeable for sugar. For the leaf drying in the dark this tissue increased 0·15 mol $O_g(KNO_3)$ and only 0·05 mol $O_g(C_{12}H_{22}O_{11})$. Just as in the case of the leaf in the light, so also here, the drying had as effect, that the permeability of the plasm increased for potassium nitrate, or decreased for sugar.

The initial $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ were lower than usual in the conducting parenchyma. The values might have been expected to be high, since this tissue usually reacted like the palisades and since it showed high values in the previous experiment, (table 6); but in no

previous test was the value so low (tables 2 and 5). As drying proceeded the O_g values increased with accompanying decrease in **i**. For the leaf exposed to the light the increase was 0·5 mol for the $O_g(KNO_3)$ and 0·45 mol for the $O_g(C_{12}H_{22}O_{11})$, so that an increase in permeability of the plasm for KNO_3, or a decrease in permeability for sugar is indicated. For the leaf in the dark the increase is a little less than for the leaf in the light. The increase is 0·2 mol in both O_g values. Since 0·2 mol KNO_3 has a higher osmotic value than 0·2 mol $C_{12}H_{22}O_{11}$, the plasm has become more permeable for KNO_3 or less permeable for sugar. Thus drying increases the permeability of the plasm for KNO_3 or decreases the permeability for sugar in this tissue, whether the leaf be exposed in the light or kept in the dark.

The spongy parenchyma, as well as the conducting parenchyma, showed lower initial O_g values than usual (cf. tables 2, 5 and 6). The O_g values increased in this tissue, as drying proceeded, for the leaf in the dark. The increments in $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ for the leaf in the light, were such that an increase in the permeability of the plasm for KNO_3 was again indicated, even though **i** decreased only within the limits of possible error.

In general, the tissues of the mesophyll indicate that the permeability of the plasm increases for potassium nitrate (or decreases for sugar), as drying proceeds, and more in the light than in the dark. These results agree with those found by TRÖNDLE, who showed that light increased the permeability of the plasm for KNO_3. (25 p. 171).

The guard cells show an increase in **i** for the leaf drying in the dark, and a decrease for the leaf drying in the light. $O_g(KNO_3)$ remained practically constant for the leaf in the dark (there was a temporary decrease of 0·05 on the second and third day), while $O_g(C_{12}H_{22}O_{11})$ increased 0·05 (there was a temporary decrease of 0·05 on the second day). For the leaf in the light $O_g(KNO_3)$ as well as $O_gC_{12}H_{22}O_{11}$) increased 0·5 mol. Here also light appears to make the plasm more permeable for KNO_3 or less permeable for $C_{12}H_{22}O_{11}$. The results obtained from the leaf in the light as well as those from the leaf in the dark, indicate increased permeability for KNO_3 in the guard cells, under the influence of drought and light, the factor of light apparently being more effective than the factor of drought.

It must not be forgotten that the effects of permeability would probably have been more pronounced, had the time of immersion not been reduced to the minimum required to plasmolyse 50% of the cells.

The purpose of these experiments is chiefly to test the reliability of the agents, while the question of variation of permeability is of subordinate importance.

Experiment III. (October 6—18, 1924)

In the second series of experiments (II_a and II_b) leaves were left to wither in the dark, while others withered in the light of an electric lamp. In this experiment a leaf supplied with water, and kept in the moist chamber (described in exp. I_a) was placed in the dark chamber (described in exp. II_a). Another leaf supplied with water, and kept in a moist chamber, was exposed to the light of the illuminating device (described in exp. II_a). In the latter case, the moist chamber consisted of a thermostat, having two of its faces made of plate glass. One of these faces was placed against the cooling tank, in such a way that the beam of light passed into the thermostat. The three other lateral faces were lined on the inside with moist filter paper, which dipped into the water, that stood an inch high on the bottom. The lid of the thermostat was also lined on the inside with moist filter paper. During the experiment the hygrometer, which was suspended inside of the moist chamber, registered 100% relative humidity at all times. The thermometer (hanging beside the hygrometer) did not vary more than 1° above or below 20° C. Fresh air (saturated with water vapor) was constantly supplied to the leaf. The general procedure of measuring O_g was the same as described in experiment II_a.

The results obtained are recorded in table 8, and the deduced i values in table 9. Since the guard cells were particularly interesting, graduation differences of 0·025 mol were employed for determining their O_g.

At the termination of the experiment the leaves were still turgescent and fresh in appearance. The increase in O_g was not considerable, when compared with the increase observed in the drying leaves in equal periods of time. The epidermal tissues of the leaf exposed to the light formed an exception in this respect and increased in both O_g values. In the check of experiment I_a and I_b (cf. table 2) a similar effect was noted in diffuse daylight, but it was not so marked as in this experiment (compare this increase with the decrease in this same tissue in experiment I_c). The initial values were more normal in these two leaves than in those chosen in the two preceding weeks (cf. table 6 and 7). The O_g of the palisades and that of the conducting parenchyma are not as high as in table 6. The spongy parenchyma has the same value as in

Table

Exp. III Simultaneous $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ Values of Leaves

		$O_g(KNO_3)$							
		Day of the							
		1	2	3	4	5	6	7	8
Leaf in the Light	Upper Epidermis	0·45	0·5	—	0·7	—	0·8	—	0·85
	Palisades	0·9	0·9	—	0·9	—	0·9	—	0·9
	Conducting Parenchyma	0·7	0·8	—	0·95	—	1·1	—	1·1
	Spongy Parenchyma	0·6	0·75	—	0·8	—	0·85	—	0·85
	Guard Cells	0·5	0·5	—	0·75	—	0·8	—	0·8
	Lower Epidermis	0·5	0·55	—	0·6	—	0·7	—	0·7
Leaf in the Dark	Upper Epidermis	0·45	0·5	—	0·55	—	0·55	—	0·55
	Palisades	0·9	0·85	—	0·8	—	0·65	—	0·55
	Conducting Parenchyma	0·7	0·7	—	0·6	—	0·55	—	0·5
	Spongy Parenchyma	0·6	0·6	—	0·5	—	0·45	—	0·5
	Guard Cells	0·5	0·55	—	0·55	—	0·5	—	0·5
	Lower Epidermis	0·5	0·55	—	0·55	—	0·55	—	0·55

Table

		$i = \frac{O_g(C_{12}H_{22}O_{11})}{O_g(KNO_3)}$			
		Day			
		1	2	3	4
Leaf in the Light	Upper Epidermis	1·33 ± 0·15	1·3 ± 0·12	—	1·22 ± 0·08
	Palisades	1·11 ± 0·07	1·11 ± 0·07	—	1·17 ± 0·07
	Conducting Parenchyma	1·22 ± 0·08	1·19 ± 0·07	—	1·11 ± 0·05
	Spongy Parenchyma	1·25 ± 0·1	1·13 ± 0·07	—	1·06 ± 0·06
	Guard Cells	1·25 ± 0·07	1·4 ± 0·07	—	1·2 ± 0·07
	Lower Epidermis	1·25 ± 0·07	1·27 ± 0·07	—	1·25 ± 0·06
Leaf in the Dark	Upper Epidermis	1·33 ± 0.15	1·3 ± 0·12	—	1·27 ± 0·1
	Palisades	1·11 ± 0·08	1·12 ± 0·07	—	1·17 ± 0·07
	Conducting Parenchyma	1·22 ± 0·09	1·22 ± 0·09	—	1·25 ± 0·1
	Spongy Parenchyma	1·25 ± 0·09	1·25 ± 0·09	—	1·3 ± 0·11
	Guard Cells	1·25 ± 0·07	1·18 ± 0·06	—	1·18 ± 0·06
	Lower Epidermis	1·25 ± 0·07	1·18 ± 0·06	—	1·27 ± 0·07

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supplied with Water and Relative Humidity 100%

$O_g(KNO_3$				$C_g(C_{12}H_{22}O_{11})$											
Test				Day of the Test											
9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
—	0·75	—	0·75	0·6	0·65	—	0·85	—	0·95	—	1·0	—	0·9	—	0·85
—	0·9	—	1·0	1·0	1·0	—	1·05	—	1·0	—	1·0	—	1·0	—	1·15
—	1·1	—	1·1	0·85	0·95	—	1·05	—	1·2	—	1·2	—	1·2	—	1·2
—	0·85	—	0·85	0·75	0·85	—	0·9	—	0·9	—	0·9	—	0·9	—	0·95
—	0·85	—	0·85	0·625	0·7	—	0·9	—	0·9	—	0·9	—	0·95	—	0·95
—	0·7	—	0·7	0·625	0·7	—	0·75	—	0·85	—	0·85	—	0·85	—	0·85
—	0·55	—	0·55	0·6	0·65	—	0·7	—	0·7	—	0·7	—	0·7	—	0·7
—	0·55	—	0·7	1·0	0·95	—	0·95	—	0·8	—	0·7	—	0·7	—	0·85
—	0·55	—	0·55	0·85	0·85	—	0·75	—	0·75	—	0·65	—	0·7	—	0·8
—	0·5	—	0·5	0·75	0·75	—	0·65	—	0·65	—	0·65	—	0·65	—	0·7
—	0·5	—	0·5	0·625	0·65	—	0·65	—	0·65	—	0·7	—	0·7	—	0·7
—	0·55	—	0·55	0·625	0·65	—	0·7	—	0·7	—	0·7	—	0·75	—	0·75

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$$i = \frac{O_g(C_{12}H_{22}O_{11})}{O_g(KNO_3)}$$

of the Test

5	6	7	8	9	10	11	12
—	1·19 ± 0·07	—	1·18 ± 0·05	—	1·2 ± 0·07	—	1·13 ± 0·07
—	1·11 ± 0·07	—	1·11 ± 0·07	—	1·11 ± 0·07	—	1·15 ± 0·03
—	1·09 ± 0·045	—	1·09 ± 0·045	—	1·09 ± 0·045	—	1·09 ± 0·045
—	1·06 ± 0·06	—	1·06 ± 0·06	—	1·06 ± 0·06	—	1·12 ± 0·07
—	1·12 ± 0·04	—	1·12 ± 0·07	—	1·12 ± 0·04	—	1·12 ± 0·04
—	1·21 ± 0·05	—	1·25 ± 0·05	—	1·21 ± 0·05	—	1·21 ± 0·05
—	1·27 ± 0·1	—	1·27 ± 0·1	—	1·27 ± 0·1	—	1·27 ± 0·1
—	1·23 ± 0·09	—	1·27 ± 0·11	—	1·27 ± 0·11	—	1·22 ± 0·09
—	1·36 ± 0·1	—	1·3 ± 0·12	—	1·27 ± 0·11	—	1·45 + 0·15
—	1·43 ± 0·16	—	1·3 ± 0·11	—	1·3 ± 0·11	—	1·4 ± 0·11
—	1·3 ± 0·07	—	1·4 ± 0·08	—	1·4 ± 0·08	—	1·4 ± 0·08
—	1·27 ± 0·07	—	1·27 ± 0·07	—	1·36 ± 0·08	—	1·36 ± 0·08

table 6 and is 0·05 higher than in table 7. The respective O_g values of the epidermal tissues and of the guard cells are about the same as in tables 6, 7 and 8.

In the upper epidermis of the leaf exposed to the light, $O_g(KNO_3)$ increased 0·4 mol in the first eight days, and decreased then until it was only 0·3 mol higher than the initial value on the twelfth day. $O_g(C_{12}H_{22}O_{11})$ showed a similar variation, increasing 0·4 mol in the first eight days, and then decreasing until the value on the twelfth day was 0·25 mol higher than the initial value. [Note that the increment in $O_g(C_{12}H_{22}O_{11})$ is lower than corresponds to the isosmotic value of the increment of $O_g(KNO_3)$].

The value of **i** decreased from 1·33 to 1.13. The increase in permeability for potassium nitrate, or the decrease in permeability for cane sugar, is evident. As regards the upper epidermis of the leaf in the dark, **i** varied only slightly and the variations in $O_g(KNO_3)$ and $O_g(C_{12}H_{22}O_{11})$ were the same.

In the lower epidermis of the leaf exposed to the light, $O_g(KNO_3)$ increased 0·2 mol, and $O_g(C_{12}H_{22}O_{11})$ increased 0·225 mol and **i** varied only slightly, decreasing from 1·25 to 1·21. The increase in permeability for potassium nitrate or a decrease in permeability for sugar (in the light), in this tissue, is not as evident as it is in the upper epidermis, but when the variations of O_g and of **i** of the same tissue in the check are taken into account, it is unmistabable. In the dark the **i** value of the lower epidermis increased from 1·25 to 1·36. $O_g(KNO_3)$ increased only 0·05 mol but $O_g(C_{12}H_{22}O_{11})$ increased 0·15 mol; evidently the lack of light made the plasm less permeable for potassium nitrate, or more permeable for sugar.

In the palisades of the leaf exposed to the light **i** increased slightly (from 1·11 to 1·15); $O_g(KNO_3)$ increased 0·1 mol and $O_g(C_{12}H_{22}O_{11})$ increased 0·15 mol. This result is exceptional and might have been caused by some error, rather than by a decrease in permeability for potassium nitrate (or an increase in permeability for sugar). The probability of error is increased by the results obtained for the check: In the dark the $O_g(KNO_3)$ of the palisades decreased 0·2 mol, while the $O_g(C_{12}H_{22}O_{11})$ decreased only 0·15 mol and **i** increased from 1·11 to 1·22, which results indicate that the lack of light decreased the permeability of the plasm for potassium nitrate (or increased the permeability for sugar).

The conducting parenchyma of the leaf in the light showed a variation of i, which indicates an increase in the permeability of the plasm for potassium nitrate (or a decrease in the permeability for sugar) (decreased from 1·22 to 1·09). The $O_g(KNO_3)$ increased 0·4 mol and $O_g(C_{12}H_{22}O_{11})$ only 0·35 mol. The variations of i and of O_g of this tissue in the check, harmonize with the conclusion arrived at, that this tissue also increases in permeability for potassium nitrate when exposed to the light (or decreases in permeability for sugar). $O_g(KNO_3)$ decreased 0·15 mol and $O_g(C_{12}H_{22}O_{11})$ decreased only 0·05 mol. The i value increased from 1·22 to 1·45.

The variations of i and O_g, noted in the spongy parenchyma, indicate the same permeability relations with exposure to light as the conducting parenchyma: $O_g(KNO_3)$ increased 0·25 mol in the light and $O_g(C_{12}H_{22}O_{11})$ only 0.2 mol, and i decreased; in the check (dark) $O_g(KNO_3)$ decreased 0·1 mol, and $O_g(C_{12}H_{22}O_{11})$ decreased only 0·05 mol and i increased from 1·25 to 1·4.

The variations of O_g and of i in the guard cells point to a less marked variation in the permeability relations, when exposed to the light, than was noted in the mesophyll tissues. For the leaf in the light $O_g(KNO_3)$ increased 0·35 mol and $O_g(C_{12}H_{22}O_{11})$ increased 0·325 mol, and i decreased from 1·25 to 1·12. For the leaf in the dark $O_g(KNO_3)$ remained practically constant, but the $O_g(C_{12}H_{22}O_{11})$ increased 0·075 and i increased. If the variations in the test and check are taken into account, it will be seen that in both leaves they indicate that light increases the permeability in the plasm for potassium nitrate (or decreases it for sugar), but in a less degree than was noted for the mesophyll tissues.

The general indications are (save for the exceptional case of the palisades of the leaf exposed to the light) that light, under the given conditions (100% relative humidity and maximum water supply), increases the permeability of the plasm for potassium nitrate (or decreases it for sugar), in all of the tissues of the lamina of the leaf.

General Conclusions from the first Method

1) The osmotic coefficients (deduced from physical data alone), decrease with the increase in the molarities. (See table 4).

2) The osmotic coefficient is not the same at all times in the same tissue, but may change with the variation of O_g and with the variation of the permeability of the plasm for potassium nitrate or sugar.

3) The osmotic value of the cell at incipient plasmolysis, and in consequence the osmotic coefficient, varied according to the cited experiments, in response to the influence of: water supply, relative humidity, light, darkness, temperature, and the various combinations of these factors. The influence does not appear to be the same in different tissues.

4) When $O_g(C_{12}H_{22}O_{11})$ is the same in different tissues, of the same leaf, at the same time, $O_g(KNO_3)$ need not be the same, and in consequence i may be different.

5) When $O_g(C_{12}H_{22}O_{11})$ is the same at different times in the same tissue, $O_g(KNO_3)$ need not be the same, and in consequence i may be different.

6) The method of measuring the variation of permeability by variations in the osmotic coefficient, cannot be relied upon to give quantitative results.

7) There are enough data on hand to indicate that in all probability, the permeability of the plasm for potassium nitrate can vary. Consequently it becomes difficult, if not impossible, to define the necessary time of immersion in a given concentration of potassium nitrate solution, so that reliable O_g values cannot be obtained.

SECOND METHOD

Determining the Degree of Plasmolysis in Tissues at Different Times

If a tissue were placed in an ideal plasmolysing agent and certain cells were plasmolysed, the cells should remain in that condition indefinitely. When 50% of the cells have become plasmolysed in an agent in a certain period of time, and at some later period more or less than that number of cells are found to be plasmolysed, the variation in the number of plasmolysed cells might result from lack of sufficient time of immersion, or from a variation in the O_g during the period of immersion.

In practice we sometimes find that less than 50% of the cells are plasmolysed, after more than that percentage had been plasmolysed, while continuously immersed in a given agent. The purpose of the tests described in this method, was to determine if O_g remains constant, while immersed in solutions of sugar or potassium nitrate. This was done by observing the percentage of plasmolysed cells after the period of immersion, which was necessary to plasmolyse 50% of the cells, and then observing at some later time if more or less than that percentage

was plasmolysed. The lower epidermis and the guard cells were chosen as subjects for these tests. In some tests solutions were employed which presumably were isosmotic with the O_g of the tissue, while in others they were more or less concentrated than that value.

The results of the tests are recorded in table 10, 11, 12 and 13. In the first row of these tables the molarities of the agents are given. The period of immersion is given in the first column. The symbol (n) indicates that less than 50% of them were plasmolysed; (p) indicates that more than 50% were plasmolysed, and (hp) indicates that 50% were plasmolysed. The sections were mounted directly on the slide, in the proper concentration of the agent, and the coverslip was surrounded with vaseline in order to prevent evaporation. In tables 10 and 11 the results for the lower epidermis are recorded, and in 12 and 13 the results for the guard cells are given. In the tests recorded in tables 10 and 12 potassium nitrate was the agent and in those recorded in tables 11 and 13 sugar was the agent.

The limiting lines in the tables, between the records showing 50% or more of the cells plasmolysed, and the records indicating less than that percentage of plasmolysed cells, have been made heavier, so that a graphic representation of the speeds of plasmolysis and of deplasmolysis resulted. By comparing table 10 with table 11 and table 12 with table 13 the difference between the speeds of plasmolysis and deplasmolysis in the tissues, when KNO_3 or $C_{12}H_{22}O_{11}$ was used, appears at a glance (care being taken to observe that the periods recorded in the first column are not the same in tables 10 and 12 as in tables 11 and 13). By comparing table 10 with table 12, and table 11 with table 13, it is evident that the reaction in the epidermal cells was not the same as that in the guard cells.

The results recorded in tables 11 and 13 are characterized by the fact (the case of concentration 0·6 table 13, forms an exception)[1], that once a tissue is plasmolysed in sugar solution, it remains plasmolysed for an indefinite length of time. The results given in table 10 and 12 are characterized by the fact, that tissues once plasmolysed in potassium nitrate solution, do not always remain plasmolysed for an indefinite length of time.

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[1] In other tests not of this series, and made on different leaves, this exceptional reaction was not observed in sugar solution, particularly not in the concentration 0·6 mol. It is possible that the section in question was abnormal.

Table 10

Variations in the Number of Epidermal Cells plasmolysed in Potassium Nitrate Solutions

(n) = less than 50%; (hp) = 50%; (p) = more than 50%

Time	Molarity											
	0·425	0·45	0·5	0·55	0·6	0·65	0·7	0·8	0·9	1·0	1·5	2·0
20″	n	n	n	n	n	n	n	n	p	p	n	n
30″	n	n	n	n	n	n	p	p	p	p	p	n
40″	n	n	n	n	n	n	p	p	p	p	p	n
50″	n	n	n	n	n	p	p	p	p	p	p	n
60″	n	hp	p	p	p	p	p	p	p	p	p	p
80″	n	p	p	p	p	p	p	p	p	p	p	p
100″	n	p	p	p	p	p	p	p	p	p	p	p
2′	n	p	p	p	p	p	p	p	p	p	p	p
3′	n	p	p	p	p	p	p	p	p	p	p	p
4′	n	p	p	p	p	p	p	p	p	p	p	p
5′	n	p	p	p	p	p	p	p	p	p	p	p
6′	n	p	p	p	p	p	p	p	p	p	p	p
7′	n	p	p	p	p	p	p	p	p	p	p	p
8′	n	p	p	p	p	p	p	p	p	p	p	p
9′	n	p	p	p	p	p	p	p	p	p	p	p
10′	n	p	p	p	p	p	p	p	p	p	p	p
15′	n	p	p	p	p	p	p	p	p	p	p	p
20′	n	n	n	p	p	p	p	p	p	p	p	p
25′	n	n	n	n	p	p	p	p	p	p	p	p
30′	n	n	n	n	p	p	p	p	p	p	p	p
40′	n	n	n	n	p	p	p	p	p	p	p	p
1h	n	n	n	n	p	p	p	p	p	p	p	p
2h	n	n	n	n	p	p	p	p	p	p	p	p
3h	n	n	n	n	p	p	p	p	p	p	p	p
4h	n	n	n	n	p	p	p	p	p	p	p	p

Table 11

Variations in the Number of Epidermal Cells plasmolysed in Sugar Solutions

(n) = less than 50%; (hp) = 50%; (p) = more than 50%

Time	Molarity											
	0·55	0·575	0·6	0·65	0·7	0·75	0·8	0·85	0·9	1·0	1·5	2·0
20″	n	n	n	n	n	n	n	n	n	n	n	n
1′	n	n	n	n	n	n	n	n	n	n	n	n
2′	n	n	n	n	n	n	n	n	n	n	n	n
3′	n	n	n	n	n	n	n	n	hp	n	n	n
4′	n	n	n	n	hp	p	p	p	p	p	n	n
5′	n	n	hp	p	p	p	p	p	p	p	n	n
6′	n	n	p	p	p	p	p	p	p	p	p	n
7′	n	n	p	p	p	p	p	p	p	p	p	n
8′	n	n	p	p	p	p	p	p	p	p	p	p
9′	n	n	p	p	p	p	p	p	p	p	p	p
10′	n	n	p	p	p	p	p	p	p	p	p	p
20′	n	n	p	p	p	p	p	p	p	p	p	p
30′	n	n	p	p	p	p	p	p	p	p	p	p
1h	n	n	p	p	p	p	p	p	p	p	p	p
2h	n	n	p	p	p	p	p	p	p	p	p	p
3h	n	n	p	p	p	p	p	p	p	p	p	p
4h	n	n	p	p	p	p	p	p	p	p	p	p

All of the tables indicate a relation between the concentration of the agent and the speed of plasmolysis. Within certain limits, the speed of plasmolysis increases with the concentration of the agent. Beyond these limits, the speed of plasmolysis decreases with the concentration of the agent. This probably results from the combined relations of the suction force and diffusion rate with the concentration of the solution. The suction force increases with the concentration, while the diffusion rate decreases with the concentration. (Cf. Results of THOVERT).

Table 12

Variations in the Number of Guard Cells plasmolysed in Potassium Nitrate Solutions

(n) = less than 50%; (hp) = 50%; (p) = more than 50%

Time	Molarity											
	0·425	0·45	0·5	0·55	0·6	0·65	0·7	0·8	0·9	1·0	1·5	2·0
20″	n	n	n	n	n	n	p	n	n	n	n	n
30″	n	n	n	n	n	p	p	p	p	p	n	n
40″	n	n	n	n	n	p	p	p	p	p	n	n
50″	n	n	n	hp	p	p	p	p	p	p	n	n
60″	n	n	n	p	p	p	p	p	p	p	n	n
80″	n	n	n	p	p	p	p	p	p	p	p	n
100″	n	n	n	p	p	p	p	p	p	p	p	n
2′	n	hp	p	p	p	p	p	p	p	p	p	n
3′	n	p	p	p	p	p	p	p	p	p	p	p
4′	n	p	p	p	p	p	p	p	p	p	p	p
5′	n	p	p	p	p	p	p	n	p	p	p	p
6′	n	p	p	p	p	n	n	n	p	p	p	p
7′	n	p	p	p	n	n	n	n	n	p	p	p
8′	n	p	n	n	n	n	n	n	n	n	p	p
9′	n	n	n	n	n	n	n	n	n	n	p	p
10′	n	n	n	n	n	n	n	n	n	n	p	p
15′	n	n	n	n	n	n	n	n	n	n	p	p
20′	n	n	n	n	n	n	n	n	n	n	p	p
25′	n	n	n	n	n	n	n	n	n	n	p	p
30′	n	n	n	n	n	n	n	n	n	n	p	p
40′	n	n	n	n	n	n	n	n	n	n	p	p
1h	n	n	n	n	n	n	n	n	n	n	n	p
2h	n	n	n	n	n	n	n	n	n	n	n	p
3h	n	n	n	n	n	n	n	n	n	n	n	p
4h	n	n	n	n	n	n	n	n	n	n	n	p

Table 13

Variations in the number of Guard Cells plasmolysed in Sugar Solutions

(n) = less than 50%; (hp) = 50%; (p) = more than 50%

Time	Molarity												
	0·55	0·575	0·6	0·65	0·7	0·75	0·8	0·85	0·9	0·95	1·0	1·5	2·0
20″	n	n	n	n	n	n	n	n	n	n	n	n	n
1′	n	n	n	n	n	n	n	n	n	n	n	n	n
2′	n	n	n	n	n	n	n	n	n	n	n	n	n
3′	n	n	n	n	n	n	n	n	n	n	n	n	n
4′	n	n	n	n	p	p	p	p	n	n	n	n	n
5′	n	n	n	n	p	p	p	p	p	n	n	n	n
6′	n	n	n	n	p	p	p	p	p	p	n	n	n
7′	n	n	n	n	p	p	p	n	p	p	p	n	n
8′	n	n	n	hp	p	p	p	p	p	p	p	p	n
9′	n	n	n	p	p	p	p	p	p	p	p	p	p
10′	n	n	hp	p	p	p	p	p	p	p	p	n	p
20′	n	n	hp	p	p	p	p	p	p	p	p	p	p
30′	n	n	hp	p	p	p	p	p	p	p	p	p	p
1h	n	n	n	p	p	p	p	p	p	p	p	p	p
2h	n	n	n	p	p	p	p	p	p	p	p	p	p
3h	n	n	n	p	p	p	p	p	p	p	p	p	p
4h	n	n	n	p	p	p	p	p	p	p	p	p	p

In table 10 it is shown that 0·425 mol KNO_3 solution fails to plasmolyse 50% of the epidermal cells; in 0·45 mol 50% are plasmolysed in 60 seconds; in 0·9 mol 50% are plasmolysed in 20 seconds (the shortest period after which the observation could be begun); in 1·5 mol 50% are plasmolysed in 30 seconds, and in 2·0 mol 50% are plasmolysed in 60 seconds. Since sugar solutions are more viscous than potassium nitrate solutions, and since they diffuse less rapidly, the differences in the speeds of plasmolysis at different concentrations are more pronounced. In table 13 it is shown that 0·575 mol fails to plasmolyse 50% of the

cells. In 0·6 mol 50% are plasmolysed in 5 minutes; in 0·9 mol in 3 minutes; in 1 mol only in 8 minutes.

Table 12 shows that 0·425 mol potassium nitrate failed to plasmolyse 50% of the guard cells; 0·45 mol plasmolysed 50% in 2 minutes (slower than for the epidermal cells—cf. table 10); 0·7 mol plasmolysed more than 50% in 20 seconds; in 0·2 mol 50% were not yet plasmolysed after 2 minutes. In sugar solution (table 13) 50% of the guard cells failed to plasmolyse in 0·575 mol; 50% were plasmolysed in 0·6 mol in 10 minutes (slower rate than was noted for the epidermal cells—cf. table 11); more than 50% were plasmolysed in 0·7 mol in 4 minutes, but 50% were not yet plasmolysed in 0·9 mol in 4 minutes; in 2·0 mol 50% were not yet plasmolysed in 8 minutes.

From table 10 it appears that beyond the concentration 0·6 mol more than 50% of the cells remained plasmolyes indefinitely in potassium nitrate solution. This behavior is not general for cells, since it is clear from table 12, that between 0·45 and 2·0 mol the speed of deplasmolysis bears roughly the same relation to the concentrations as does the speed of plasmolysis. In table 10 it is shown that from 0·45 mol potassium nitrate to 0·55 mol, the epidermal cells increased their speed of deplasmolysis with the concentration. In table 12 it is shown, that from 0·45 mol potassium nitrate to 0·8 mol, the guard cells increase their speed of deplasmolysis with the concentration, but beyond this concentration, the speed of deplasmolysis decreases with the concentration, and more than 50% of the guard cells remained indefinitely plasmolysed in a 2·0 mol solution.

General Conclusions from the Second Method

1) Potassium nitrate solutions plasmolyse more rapidly than corresponding concentrations of sugar solutions.

2) In both agents the speed of plasmolysis increases with the concentration up to a certain limit, after which the rate decreases with the concentration.

3) In potassium nitrate solutions, cells deplasmolyse. For the guard cells the rate of deplasmolysis increases with the concentration up to a certain limit, after which the rate decreases with the concentration. For the epidermal cells deplasmolysis increases with the concentration up to a certain limit of concentration, after which some cells remain plasmolysed indefinitely.

4) It is possible that the O_g of a cell increases during immersion in a potassium nitrate solution which is isotonic with the original O_g of the cell. It follows that, it is possible that a potassium nitrate solution, which is isotonic with the original O_g of the cell, will fail to plasmolyse.

5) If plasmolysis is to be observed in 50% of the cells, when employing potassium nitrate as an agent, the observation must be made within definite limits of time, which vary with the concentration of the solutions employed, and the nature of the tissue that is tested.

6) When potassium nitrate is employed as an agent, it is difficult to state a definite time in which the observation is to be made, to obtain an approximation of the O_g value.

7) It is easy to determine the time in which observations should be made to read the best approximations of O_g, when sugar is employed as an agent.

8) The determinations of $O_g(C_{12}H_{22}O_{11})$ are more reliable than those of $O_g(KNO_3)$.

THIRD METHOD

Determining the Degree of Plasmolysis in Cells at Different Times.

If a cell were placed in an ideal agent and were plasmolysed, it should remain plasmolysed for an indefinite length of time. Sometimes it happens, when employing certain agents, that a cell which has shown a maximum degree of plasmolysis at a certain time, manifests a reversal of the process (deplasmolysis) at a later time.

It is usually assumed that solute enters the cell and increases the O_g, so that water is drawn back into the cell. This explanation seems plausible and probably it is correct in many cases. Another explanation is that the cell produces within itself, osmotically active substances (anatonosis), which increase the O_g.

To test the degree of plasmolysis in cells at different times two methods were followed: (a) Drawings were made of the relative positions of the plasm and these were compared. (b) Records were kept of the time necessary to reach maximum plasmolysis and deplasmolysis.

(a) Determinations by Drawings.

The ivy leaves were chosen in the usual manner from the same plant as in all previous tests. Only fresh leaves were tested. The sections were mounted directly on the slide as previously described, and

the preparation was sealed with vaseline. They were mounted in various concentrations of potassium nitrate and of sugar solutions, and kept under constant observation, tracing the cell walls and the positions of the film with the aid of a drawing apparatus.

When sugar solutions were employed the drawings for all of the various tissues of the lamina showed that, when maximum plasmolysis was reached in any cell, its position was maintained during the entire period of observation, which was more than an hour.

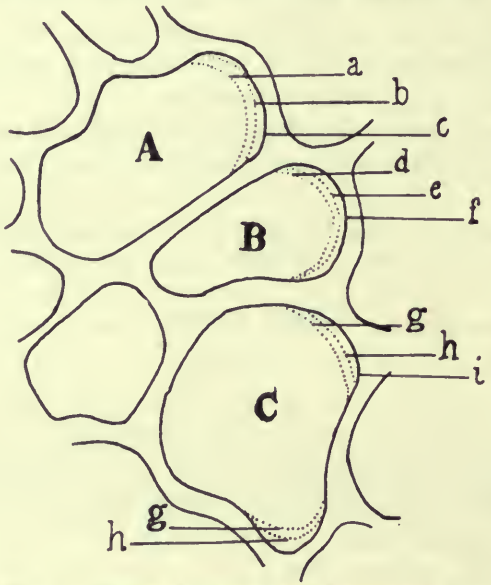

Fig. 1. Stages of plasmolysis in epidermal cells

When potassium nitrate solutions were employed, the drawings frequently showed that cells, from any of the various tissues of the lamina, after reaching a maximum degree of plasmolysis, deplasmolysed partially or completely.

For a group of cells immersed in potassium nitrate solution, the time to reach maximum plasmolysis is not the same for the different cells, nor does deplasmolysis set in at the same time in all of them. Perhaps the osmotic value is different in the various cells; or perhaps there is an increase in the osmotic value which is not the same for all of them; or the fact, that they are of different capacities, causes this difference in reaction in the various members of the group.

Only two of the drawings are reproduced here. Fig. 1 shows one of the drawings taken from the lower epidermis. Fig. 2, one of the drawings from the spongy parenchyma.

In fig. 1, the cells **A**, **B**, and **C** were plasmolysed in a 0·525 mol potassium nitrate solution, while the fourth cell failed to plasmolyse. **A** reached maximum plasmolysis (position **a**) in 3 minutes; **B** (position **d**) in 4 minutes: and **C** (position **g**) in 8 minutes. Deplasmolysis progressed faster in **A** and **B** than in **C**. The position **b** (in **A**) was reached after 5 minutes; and position **e** (in **B**) after 6 minutes; and position **h**

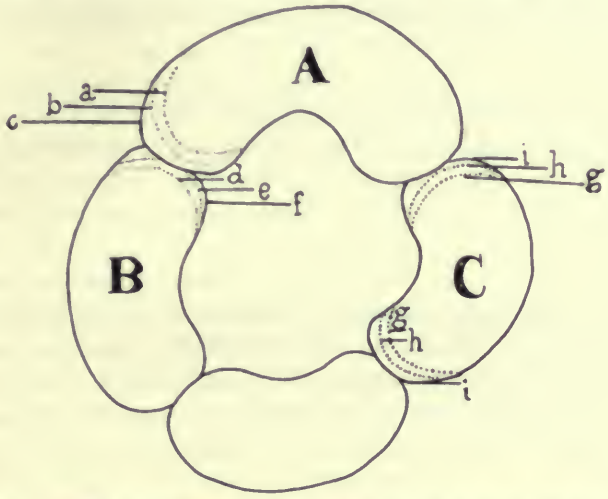

Fig. 2. Stages of plasmolysis in the cells of the spongy parenchyma

(in **C**) only after 12 minutes. **A** was completely deplasmolysed (position **c**) after 7 minutes; **B** (position **f**) after 9 minutes; and **C** (position **i**) only after 15 minutes.

In the group of cells taken from the spongy parenchyma (fig. 2), **A**, **B**, and **C** showed maximum plasmolysis after 2·5 minutes in 0·675 mol potassium nitrate solution (position **a**, **d** and **g**). The position **e** (in **B**) was reached after 5 minutes and **B** was completely deplasmolysed (position **f**) after 10 minutes. Position **b** (in **A**) was reached only after 10 minutes, and **A** was deplasmolysed only after 15 minutes. Position **h** (in **C**) was reached after 10 minutes and **C** was deplasmolysed only after 20 minutes.

In other tissues similar differences were found. For instance, in a stomatal complex it happened frequently (immersed in potassium nitrate solution), that one guard cell deplasmolysed after a few minutes, while the other failed to do so after an hour. HOEFLER found similar relations to exist in neighboring cells of the same tissue and says that the permeability can be different in them. (**11** p. 442).

When sugar solution was employed as an agent all of the drawings showed, that the cells of all of the tissues remained plasmolysed, and showed no traces of a reversal of the process, while remaining in the solution that plasmolysed them. When the film reached the position of maximum contraction, this position was maintained for an indefinite length of time.

General Conclusions from the Results of Method (a).

1) Adjacent cells of the same tissue may have different O_g values.

2) Cells can deplasmolyse in the same solution of potassium nitrate which plasmolysed them. The deplasmolysis may be complete or partial.

3) Cells of the same tissue do not usually plasmolyse at the same speed, and if deplasmolysis sets in, the speed of deplasmolysis is not usually the same in all of the cells of a given tissue.

4) For the cells of a given group, chosen from a given tissue of the ivy leaf, and immersed in a solution of potassium nitrate, no simple relation exists between the speed of plasmolysis and the speed of deplasmolysis.

5) It is difficult to state the most favorable time in which $O_g(KNO_3)$ should be measured for a given tissue, but it is easy to state the time in which reliable $O_g(C_{12}H_{22}O_{11})$ values can be obtained for a given tissue.

6) When a section is immersed in a potassium nitrate solution for a greater or less period of time than is necessary to plasmolyse the tissue, serious error in the measurement of O_g may result. The degree of this error cannot be estimated.

(b) Determination by Time Records.

In section (a) it was noted (by the drawings), when cells were placed in a solution of potassium nitrate, and a maximum degree of plasmolysis was attained, that this condition of maximum plasmolysis did not persist. In some cases deplasmolysis was complete, in other cases only partial. In the tests described in this section records were

kept of the time when plasmolysis began, when the maximum degree of contraction was reached, when deplasmolysis began and when it was completed. The results are given in tables 14 and 15. First fresh ivy leaves (chosen from the usual place) were employed, and after a number of hours of drying (indicated in the third column of the tables) the same were used again. The tests were always made on a single cell, which was constantly kept in the field of observation (as previously described). The O_g of the tissue was first measured in the usual manner; the molar value is indicated in the fourth column. The concentration of the agent employed was generally of the same osmotic value as the O_g of the tissue, but sometimes a little greater (see fifth column). When the time to be recorded was more than a certain number of hours the sign (∞) was employed, e. g. in table 14 in the first trial on a cell of the upper epidermis, the cell failed to deplasmolyse completely after several hours, which is indicated in the last column by the (∞) sign.

In table 14 are the records for the tests with potassium nitrate solution as agent, and in table 15 the records for the tests when sugar solutions were employed.

Previously it was concluded that cells of the same tissue do not all have the same O_g. From this it is clear, how a given cell can still be plasmolysed in a solution having a molar value less than the O_g of the tissue; and also how another cell (e. g. upper epidermis, 9th trial, table 14) may not be excessively plasmolysed in a solution having a molar value greater than the O_g of the tissue.

The most remarkable thing about the results obtained and recorded in table 14 is the irregularity of the reactions in cells of the same tissue[1]. (See column 6 to 9). Sometimes deplasmolysis occurred very quickly and completely (e. g. upper epidermis, 12th trial), and at other times slowly and only partially (e. g. upper epidermis, 1st trial or 20th trial). The trials on the upper epidermis from the 5th to the 9th are particularly impressive in this respect, because the number of hours of drying was the same; in all of them; the concentration of the potassium nitrate solution was the same; and the O_g of the tissue, of course, was the same. In the seventh and eighth trials, for instance, plasmolysis began in the same time, maximum plasmolysis was reached in the same time, and

[1] In tables 14 and 15, the trials for a given tissue were always made on the same leaf, e. g. 1 to 20 for the upper epidermis, table 14.

Table 14

Record of the Speeds of Plasmolysis and Deplasmolysis in the Cells of Various Tissues, Immersed in Potassium Nitrate Solution

Tissue from which the Cell was chosen	Trial Number	Hours of Drying	O_g of the Tissue	Molarity of the Agent	Plasmolysis began after	Maximum Plasmolysis reached after	Deplasmolysis began after	Deplasmolysis completed after
Upper Epidermis	1	0	0·525	0·525	3′	10′	30′	∞
	2	24	0·5	0·5	3′	12′	18′	25′
	3	24	0·5	0·525	2′	10′	15′	20′
	4	48	0·55	0·55	2′	10′	15′	20′
	5	48	0·525	0·525	1′	4′	5′	25′
	6	48	0·5	0·525	1′	3′	5′	17′
	7	48	0·5	0·525	2′	5′	6′	∞
	8	48	0·5	0·525	2′	5′	6′	15′
	9	48	0·5	0·525	2′	5′	7′	19′
	10	48	0·5	0·525	2′	5′	9′	∞
	11	72	0·525	0·525	2′	5′	7′	15′
	12	96	0·525	0·525	1′	3′	4′	7′
	13	96	0·525	0·525	2′	4′	6′	9′
	14	96	0·525	0·525	4′	8′	10′	14′
	15	120	0·375	0·375	2′	6′	11′	∞
	16	144	0·35	0·35	2′	7′	12′	16′
	17	144	0·35	0·35	2′	7′	10′	12′
	18	144	0·35	0·35	2′	7′	13′	∞
	19	168	0·65	0·65	2′	6′	10′	18′
	20	192	0·5	0·5	2′	7′	11′	∞
Conducting Parenchyma	1	0	0·9	0·9	1′	3′	9′	14′
	2	0	0·9	0·9	2′	4′	11′	16′
	3	24	0·975	0·975	1′	3′	8′	14′
Palisades	1	0	0·9	0·9	6′	10′	∞	∞
	2	0	0·9	0·9	10′	14′	∞	∞
	3	0	0·9	0·9	15′	18′	∞	∞
	4	48	1·075	1·075	8′	15′	∞	∞
	5	72	1·25	1·25	8′	16′	∞	∞
	6	96	1·5	1·5	3′	4′	∞	∞
Spongy Parenchyma	1	0	0·675	0·675	40″	2′	2·5′	15′
	2	0	0·675	0·675	40″	2′	2·5′	10′
	3	0	0·675	0·675	40″	2′	2·5′	20′
	4	24	0·825	0·825	40″	3′	3·5′	∞
	5	24	0·825	0·825	40″	3′	3·5′	∞
	6	24	0·825	0·825	10′	16′	25′	∞
	7	72	1·1	1·1	30″	3′	4′	8′
	8	72	1·1	1·1	30″	3′	5′	∞
	9	72	1·1	1·1	30″	3′	5′	∞
	10	96	1·3	1·3	30″	3′	6′	20′

Table 14 (Continued)

Tissue from which the Cell was chosen	Trial Number	Hours of Drying	O_g of the Tissue	Molarity of the Agent	Plasmolysis began after	Maximum Plasmolysis reached after	Deplasmolysis began after	Deplasmolysis completed after
Lower Epidermis	1	0	0·625	0·625	2·5′	8′	18′	25′
	2	0	0·625	0·625	2·5′	8′	15′	∞
	3	0	0·625	0·625	2·5′	8′	15′	∞
	4	24	0·65	0·65	2·5′	7′	18′	25′
	5	24	0·65	0·65	2·5′	8′	15′	∞
	6	72	0·625	0·625	2·5′	8′	15′	25′
	7	72	0·625	0·625	2·5′	9′	16′	∞
Guard Cells	1	0	0·65	0·65	2′	3′	4′	6′
	2	0	0·65	0·65	1·5′	3′	4′	7′
	3	24	0·7	0·7	1·5′	3′	4′	6·5′
	4	24	0·7	0·7	1·5′	3′	4′	7′
	5	72	0·7	0·7	1·5′	3′	4′	6′
	6	72	0·7	0·7	2′	3′	4′	7′

deplasmolysis began in the same time, but while deplasmolysis was completed after 15 minutes in the 8th trial, it remained partial for more than several hours in the 7th trial. Similar irregularities were noted in the cells of the lower epidermis.

All of the palisade cells tested, failed to deplasmolyse after long immersion (several hours) in the agent. This phenomenon is probably not general, since at other times, in experiments with other ivy leaves, palisade cells were noted to deplasmolyse completely in the potassium nitrate solution which had plasmolysed them.

The trials made with guard cells, contrast strongly with those made with palisade cells. The guard cells deplasmolysed quickly in all of the trials[1].

Only three trials were made with conducting-parenchyma cells, but all three resemble each other in their results. They plasmolysed quickly and deplasmolysed completely in about 15 minutes.

The spongy parenchyma (which ordinarily resembles the palisade tissue in its reaction) shows the same irregularity in the reactions of its individual cells as the epidermal tissues. Deplasmolysis occurred very quickly in some cases, and not at all in others.

[1] It will be recalled that in previous tests on ivy leaves, guard cells sometimes failed to deplasmolyse.

There seems to be no relation between the time necessary to introduce the first phase of plasmolysis, and the time necessary to complete deplasmolysis in the cell. The irregularities noted in the time necessary to observe the various phases of plasmolysis and deplasmolysis, appear surprising at first; when it is recalled that the speed of plasmolysis and the speed of deplasmolysis in a single cell, depends upon many factors, which can be combined and permuted in many ways, the irregularities are to be expected. Some such influencing factors would be, the capacity of the cell, the elastic constants of the cell wall, the degree of saturation of the cell at the time of the test, the difference in the osmotic value of the contents of the cell and the osmotic value of the agent, the permeability of the plasm, the tension and pressure exerted by the neigboring cells when some cells increase or decrease their volume, and the diffusion rate of the agent. The influence exerted upon the speed of plasmolysis and the speed of deplasmolysis, depends upon the particular combination of the factors, as well as upon the sum of their individual effects.

There can be no doubt, but that the O_g of the cells is changed when the section is immersed in potassium nitrate solution. This change cannot be caused by the extraction of water, for previous tests, and the tests recorded in table 15 (which will be described below), all fail to show a variation in O_g when the section is immersed in sugar solution, even though it also extracts water from the cells. It is not probable that the potassium nitrate can cause molecular changes in the cell without entering it.

While it seems certain from all of the data thus far obtained, that the plasm of the tissues studied is permeable for potassium nitrate, no definite conclusions can be drawn from the data regarding the relative permeability in cells of the same tissue or cells of different tissues. The speed of plasmolysis and the speed of deplasmolysis fail as adequate indicators of the relative permeability, since they depend not only on permeability, but also upon many other factors.

All of the results of the tests (recorded in table 15) in which sugar solution was employed as a plasmolysing agent, lead to the conclusion, [also arrived at by method (a)] that a cell which is plasmolysed in this agent, fails to deplasmolyse when constantly kept in the same concentration of sugar solution.

Cells of the same tissue begin to plasmolyse in about the same time (column 6), but the speeds of plasmolysis of the cells of different tissues vary widely.

Table 15.

Record of the Speeds of Plasmolysis and Deplasmolysis in the Cells of Various Tissues, Immersed, in Sugar Solution.

Tissue from which the Cell was chosen	Trial Number	Hours of Drying	O_g of Tissue	Molarity of the Agent	Plasmolysis began after	Maximum Plasmolysis reached after	Plasmolysis Constant for
Upper Epidermis	1	0	0·675	0·675	20′	40′	16h
	2	72	0·675	0·7	20′	40′	16h
	3	168	0·85	0·85	20′	60′	14h
Conducting Parenchyma	1	0	1·05	1·05	5′	8′	16h
	2	24	1·125	1·125	3′	6′	16h
Spongy Parenchyma	1	0	0·775	0·775	10′	30′	16h
	2	24	0·85	0·85	10′	30′	16h
	3	48	0·95	0·95	8′	25′	16h
Lower-Epidermis	1	0	0·625	0·625	18′	36′	16h
	2	72	0·625	0·625	20′	38′	16h
Guard Cells	1	0	0·65	0·65	2′	7′	14h
	2	72	0·7	0·7	2′	8′	15h
	3	96	0·7	0·7	2′	15′	14h

It is worthy of notice, that the guard cells showed the first phase of plasmolysis shortly after the immersion of the section. They reached maximum plasmolysis in less than 20 minutes.

From the results recorded in table 15, it is evident that a reliable approximation of the true O_g of any tissue can be obtained, if the measurement is made between 35 and 45 minutes, and if for any reason it is desired to leave the sections immersed in the solution for an hour or more, it can be done without great risk of change in the O_g. (My observations were made on a tissue for 14, 15, or 16 hours. See column 8). Since other factors, which are independent of the nature of the agent employed, might enter to vary the O_g of a cell, it is advisable to operate as quickly as possible and not to extend the time of immersion beyond reasonable limits.

General Conclusions from the Results of Method (b).

1) Cells of the same tissue do not always plasmolyse with the same speed, in concentrations of the agent which is sufficient to plasmolyse them.

2) Not all of the cells, which are plasmolysed in a given concentration of potassium nitrate solution, deplasmolyse completely while immersed in the same solution.

3) Cells of the same tissue, which deplasmolyse while remaining immersed in the same solution of potassium nitrate that plasmolysed them, do not all deplasmolyse at the same rate. (Cf. **12** p. 326).

4) Cells which have been plasmolysed with a sugar solution, remain plasmolysed for an indefinite length of time while immersed in the same solution (so long as no factor which is independent of the agent enters to alter the O_g).

5) It is difficult, and probably impossible to determine the most favorable time of immersion for the measurement of $O_g(KNO_3)$.

6) The $O_g(C_{12}H_{22}O_{11})$ of the tissues studied, can be measured after the section has been immersed in the sugar solution for 20 minutes. No serious error in the O_g reading will result if the period of immersion be extended within reasonable limits.

7) Cane sugar is a more reliable plasmolysing agent than potassium nitrate, for determining the O_g of cells and tissues.

FOURTH METHOD

Observing Variations in the Degree of Plasmolysis in a Cell, after Successive Immersions in Sugar Solution and Potassium Nitrate Solution

Epidermal cells and guard cells were employed in these tests. Leaves were chosen from the same ivy plant that furnished material for all of the previous tests. Only fresh leaves were used.

The $O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$ (see column 2, tables 16 and 17) were first determined for the tissue, and then the $O_g(C_{12}H_{22}O_{11})$ of the cell which was chosen for study, was determined on a new section (4th column of the tables).

Since the cell chosen for study had to be found quickly after being dismounted and remounted, a method of localizing the cell had to be devised. The problem was similar to the localization of a point by means of an ordinate and abscissa, so that the method of coördinates was followed. With a pair of very fine dividers, two holes were pierced into the tissue. They lay close together and appeared simultaneously in the field of vision during microscopic examination. About ten intact cells intervened. A cover-slip, upon which had been drawn a horizontal line and several others perpendicular to it, was placed on the diaphragm of the eye-piece. The intersection of a vertical line with the horizontal

Table 16.

Variations in the Degree of Plasmolysis in Epidermal Cells after Successive Immersions in Sugar and Potassium Nitrate Solutions.

(n) = Deplasmolysed, Pl(+) = Increase, Pl(−) = Decrease in Plasmolysis.

Serial Number of Test	$O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$ of the Tissue	Number of Cell	$O_g(C_{12}H_{22}O_{11})$ of Cell	Period of Immersion in $C_{12}H_{22}O_{11}$	Molarity of KNO_3 Solution	First Phase	Last Phase
I	0·625 and 0·45	1	0·625	30′	0·45	Pl(+) in 5′	Constant for 30′
		2	0·625	30′	0·45		
II	0·625 and 0.45	1	0·625	30′	0·45	Constant for 3′	Pl(−), (n) after 8′
		2	0·625	30′	0·45		
		3	0·625	30′	0·45		
		4	0·625	30′	0·45		
		5	0·625	30′	0·45		Pl(−), (n) after 12′
		6	0·625	30′	0·45		
		7	0·625	30′	0·45		
III	0·625 and 0·45	1	0·65	45′	0·525	Pl(+)	Pl(+), (n) after 30′
		2	0·65	45′	0·525		
		3	0·65	45′	0·525		
		4	0·65	45′	0·525		
		5	0·65	45′	0·525		
		6	0·65	45′	0·525		
		7	0·65	45′	0·525		
		8	0·65	45′	0·525		
IV	0·625 and 0.45	1	0·625	1 h	0·475	Constant for 4′	Pl(−), (n) after 10′
		2	0·625	1 h	0·475		
		3	0·625	1 h	0·475		
		4	0·625	1 h	0·475		Pl(−), (n) after 15′
		5	0·625	1 h	0·475		
		6	0·625	1 h	0·475		
V	0·625 and 0·45	1	0·625	1 h	0·45	Constant for 3′	Pl(−), (n) after 15′
		2	0·625	1 h	0·45		
		3	0·625	1 h	0·45		
		4	0·625	1 h	0·45		
		5	0·625	1 h	0·45		
		6	0·625	1 h	0·45		
		7	0·625	1 h	0·45		
		8	0·625	1 h	0·45		
VI	0·625 and 0·45	1	0·675	1·5 h	0·5	Pl(+) for 10′	Pl(−), (n) after 30′
		2	0·675	1·5 h	0·5		
		3	0·675	1·5 h	0·5		

Table 16 (Continued)

Serial Number of Test	$O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$ of the Tissue	Number of Cell	$O_g(C_{12}H_{22}O_{11})$ of Cell	Period of Immersion in $C_{12}H_{22}O_{11}$	Molarity of KNO_3 Solution	First Phase	Last Phase
VII	0·625 and 0·45	1	0·675	2 h	0·525	Pl(+) for 12′	Pl(—), (n) after 30′
		2	0·675	2 h	0·525		
		3	0·675	2 h	0·525		
		4	0·676	2 h	0·525		
		5	0·675	2 h	0·525		
VIII	0·625 and 0·45	1	0·675	1·5 h	0·5	Pl(+) for 5′	Pl(—), (n) after 30′
		2	0·675	1·5 h	0·5		
		3	0·675	1·5 h	0·5		
		4	0·675	1·5 h	0·5		
		5	0·675	1·5 h	0·5		
		6	0·675	1·5 h	0·5		
IX	0.625 and 0·45	1	0·675	2 h	0·575	Pl(+) for 3′	Pl(—), not (n) after 1 h
		2	0·675	2 h	0·575		
		3	0·675	2 h	0·575		
		4	0·675	2 h	0·575		

was made to coincide with one of the holes in the section, and the horizontal line passed from one hole to the other. The desired cell was located by counting its order along the horizontal line, or along some vertical line. This arrangement was found better than using a regular grating, placed in the eye-piece, since there were but few lines in the field of vision and the definition of the film of plasm was better. The many meshes of the grating destroy the contrast of the image in the field of vision.

When the section had been immersed in sugar solution, for the period of time indicated in the 5th column of the tables, it was quickly washed in potassium nitrate solution of the same concentration as that in which it was to be mounted. After mounting the section in potassium nitrate solution, the coverslip was surrounded with vaseline. The concentration of the potassium nitrate solution, in which the section was mounted, was sometimes equal, and at other times greater, than the estimated $O_g(KNO_3)$ of the cell. Immediately after mounting the section, the variations of the position of the film was observed. The first and last phases of this variation were recorded in the tables. When plasmolysis increased, this was indicated by the symbol Pl (+), when it

decreased it was indicated by Pl (—). Observation was never begun later than one minute after the final mounting.

Each series of tests as recorded in the tables, was taken from a fresh leaf. The serial number is given in the first column. The O_g of the tissue is given in the second column. The number of the particular cell in a series is given in the third column. The length of time for which the section was immersed in sugar solution in order to determine the $O_g(C_{12}H_{22}O_{11})$ of the cell (fourth column), is given in the fifth column. The concentration of the potassium nitrate solution employed for the final test, is given in the sixth column. The first phase of the variation of the position of the film, is given in the seventh column, and the last phase in the eighth column. The time of observation never extended beyond 30 minutes.

The results for the tests on the lower epidermis are recorded in table 16, and those for the guard cells in table 17.

If the determined $O_g(C_{12}H_{22}O_{11})$ and the estimated $O_g(KNO_3)$ are isosmotic values, and if the O_g of the cell does not vary as the result of the penetration of the solute when immersed in the potassium nitrate

Table 17

Variations in the Degree of Plasmolysis in Guard Cells, after Successive Immersions in Sugar and Potassium Nitrate Solutions.

(n) = Deplasmolysed, Pl(+) = Increase, Pl(—) = Decrease in Plasmolysis.

Serial Number of Test	$O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$ of the Tissue	Number of Cell	$O_g(C_{12}H_{22}O_{11})$ of the Cell	Period of Immersion in $C_{12}H_{22}O_{11}$	Molarity of KNO_3 Solution	First Phase	Last Phase
I	0·65 and 0·525	1	0·625	30′	0·6	Pl(+) for 3′	Pl(—), (n) after 20′
		2	0·625	30′	0·6		
		3	0·625	30′	0·6		
		4	0·625	30′	0·6		
		5	0·625	30′	0·6		
II	0·65 and 0·525	1	0·675	45′	0·525	Constant for 2′	(n) after 4′
		2	0·675	45′	0·525		
		3	0·675	45′	0·525		
		4	0·675	45′	0·525		
		5	0·675	45′	0·525		
		6	0·675	45′	0·525		
		7	0·675	45′	0·525		
		8	0·675	45′	0·525		

Table 17 (Continued)

Serial Number of Test	$O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$ of the Tissue	Number of Cell	$O_g(C_{12}H_{22}O_{11})$ of the Cell	Period of Immersion in $C_{12}H_{22}O_{11}$	Molarity of KNO_3 Solution	First Phase	Last Phase
III	0·65 and 0.525	1	0·65	1 h	0·525	Constant for 2′	Pl(—), not (n) after 30′
		2	0·65	1 h	0·525		
		3	0·65	1 h	0·525		
IV	0·65 and 0.525	1	0·625	1 h	0·475	Constant for 4′	Pl(—), (n) after 10′
		2	0·625	1 h	0·475		
		3	0·625	1 h	0·475		
		4	0·625	1 h	0·475		Pl(—), (n) after 15′
		5	0·625	1 h	0·475		
		6	0·625	1 h	0·475		
		7	0·625	1 h	0·475		
V	0·625 and 0·45	1	0·675	1·5 h	0·5	Pl(—)	(n) after 3′
		2	0·675	1·5 h	0·5		„ „ 2′
		3	0·675	1·5 h	0·5		„ „ 2′
		4	0·675	1·5 h	0·5		„ „ 20′
		5	0·675	1·5 h	0·5		„ „ 25′
		6	0·675	1·5 h	0·5		„ „ 30′
VI	0·65 and 0.525	1	0·675	2 h	0·525	Pl(—)	(n) after 2′
		2	0·675	2 h	0·525		
		3	0·675	2 h	0·525		
		4	0·675	2 h	0·525		
		5	0·675	2 h	0·525		
		6	0·675	2 h	0·535		
		7	0·675	2 h	0·545		
		8	0·675	2 h	0·525		
VII	0·65 and 0.525	1	0·675	2 h	0·575	Pl(—)	(n) after 3′
		2	0·675	2 h	0·575		
		3	0·675	2 h	0·575	Constant for 5′	Pl(—), (n) after 30′
		4	0·675	2 h	0·575	Constant for 5′	Pl(—), (n) after 30′
		5	0·675	2 h	0·575	Constant for 2′	Pl(—), (n) after 15′
		6	0·675	2 h	0·575	Constant for 2′	Pl(—), (n) after 10′
		7	0·675	2 h	0·575	Constant for 5′	Pl(—), (n) after 40′
		8	0·675	2 h	0·575	Pl(—)	(n) after 2′
VIII	0·65 and 0·525	1	0·675	30′	0·6	Pl(+) for 10′	Constant for 30′
		2	0·675	30′	0·6		
		3	0·675	30′	0·6		
		4	0·675	30′	0·6		
		5	0·675	30′	0·6		
		6	0·675	30′	0·6		
		7	0·675	30′	0·6		
		8	0·675	30′	0·6		

solution, and if no molecular changes occur in the contents of the cell that would change O_g, then the degree of plasmolysis should remain constant after the cell is carried from the sugar solution into the potassium nitrate solution. The nearest approach to such a result is found in series I table 16, for the lower epidermal cells, and series III table 17 for the guard cells. The cell and the tissue had the same O_g values in these cases, and the potassium nitrate solution, in which the cell was immersed, was as closely isosmotic with the $O_g(C_{12}H_{22}O_{11})$ as could be estimated. (Compare columns 2, 4, and 6.) For the lower epidermis the degree of plasmolysis increased for five minutes and then remained constant for 30 minutes, after which observation ceased. In the guard cells there was no change for 2 minutes, after which plasmolysis decreased for five minutes and then remained constant for 30 minutes, after which observation ceased.

In series II table 16 quite a different result is noted under approximately the same conditions. In the first three minutes there was no change but later, deplasmolysis set in and progressed rapidly. As previous and succeeding tests showed, the increase in the O_g of the cell (indicated by the deplasmolysis) cannot be due to the extraction of water by the sugar. Induction of molecular changes by the potassium nitrate without solute entering the cell, is most improbable. Probably solute enters the cell and very rapidly increases the O_g of the cell. It is possible that the potassium nitrate upon entering the cell, causes molecular changes in the contents that tend to increase the O_g value.

When a more concentrated value of potassium nitrate was chosen than that which was isosmotic with $O_g(C_{12}H_{22}O_{11})$ (e. g. series IV, table 16), the results were similar to the results just described for series II table 16.

It will be noticed upon examining the various results, that a variation in the time of immersion in the sugar solution (column 5), does not cause any noticeable difference in the reactions.

The results also show, what has been previously concluded from the results given in table 14, that different cells in the same tissue do not always react in the same way, when immersed in the same solution of potassium nitrate.

General Conclusions from the Results of the Fourth Method

1) In a cell that had been plasmolysed in a sugar solution, and was then placed in an approximately isosmotic potassium nitrate solution, a balanced condition was never reached immediately.

2) The reaction observed in a cell, which was plasmolysed in a sugar solution, and which was then placed in an approximately isosmotic potassium nitrate solution, sometimes resembled the reaction in another cell (from the same tissue) which was transferred from the plasmolysing sugar solution to a hypertonic potassium nitrate solution.

3) The immersion of a cell in a sugar solution that causes incipient plasmolysis, for a period of time that is considerably greater than the time that is necessary to plasmolyse, does not noticeable vary the reaction in the cell when placed in potassium nitrate solution (e. g. compare series II and V, table 16; or series II and VI, table 17).

4) After being plasmolysed in a sugar solution, cells of the same tissue do not always react in the same way when placed in a potassium nitrate solution. (Compare cell No. 1 and cell No. 6, series V, table 17).

5) It is possible that the O_g of a cell is increased when it is placed in a solution of potassium nitrate which is of equal or of greater concentration than the value which is isosmotic with $O_g(C_{12}H_{22}O_{11})$.

FIFTH METHOD

Determining the Variation in the $O_g(C_{12}H_{22}O_{11})$ in a Cell, after Immersion in a Potassium Nitrate Solution

The lower epidermis and the guard cells, taken from fresh ivy leaves, were used for these tests. Each series of tests was made on a different leaf.

After $O_g(C_{12}H_{22}C_{11})$ and $O_g(KNO_3)$ were determined for the tissue, a fresh section was placed in a sugar solution for 40 minutes. Sometimes this sugar solution was of the same concentratien as the $O_g(C_{12}H_{22}C_{11})$ value of the tissue (e. g. table 18, series III), and at other times it was greater (e. g. table 10, series IV), or less (e. g. table 18, series II) than the $O_g(C_{12}H_{22}O_{11})$ value of the tissue. At the end of the 40 miuutes, the section was examined and a plasmolysed cell was located in the manner previously described. The section was then placed in a potassium nitrate solution for 10 minutes. The concentration of this solution was equal to, or less than, the estimated $O_g(KNO_3)$ value of the cell. After this treatment the section was placed in a sugar solution for 35 minutes. If the cell in question was deplasmolysed at the end of this time, its O_g was evidently greater than the concentration of the sugar solution last employed. This is indicated by placing a (+) sign in back of the concentration of the sugar solution last employed (see tables 18 and 19, last column, and read „greater than“ the number given).

The results obtained by this method are recorded in tables 18 and 19.

Table 18

Variations in $O_g(C_{12}H_{22}O_{11})$ of Epidermal Cells, after Immersion in a Potassium Nitrate Solution

(0·a +) = more than 0·a.

Serial Number of the Test	$O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$ of the Tissue	Number of the Cell	$O_g(C_{12}H_{22}O_{11})$ of the Cell	Molarity of the KNO_3 Solution	Finale $O_g(C_{12}H_{22}O_{11})$ of the Cell
I	0·625 and 0·45	1	0.6	0·3	0·6 +
		2	0.6	0·3	0·6 +
II	0·625 and 0·45	1	0·6	0·3	0·625 +
		2	0·6	0·3	0·625 +
		3	0·6	0·3	0·625 +
		4	0·6	0·3	0·625 +
III	0·625 and 0·45	1	0·625	0·35	0·675 +
		2	0·625	0·35	0·675 +
IV	0·625 and 0·45	1	0·65	0·4	0·7 +
		2	0·65	0·4	0·7 +
		3	0·65	0·4	0·7 +
		4	0·65	0·4	0·7
V	0.625 and 0·45	1	0·675	0·45	0·725 +
		2	0·675	0·45	0·725 +
		3	0·675	0·45	0·725 +
		4	0·675	0·45	0·725 +
		5	0·675	0·45	0·725 +

In only one case was the test cell not deplasmolysed after the treatment (table 18, series IV No. 4). The increase in O_g could not have been due to the treatment in sugar solution, for all previous tests show that cells can remain in sugar solution much longer than 35 minutes without showing a variation in thee degree of plasmolysis. From this it is evident that a potassium nitrate solution which is approximately of the same concentration as the $O_g(KNO_3)$ value, or even considerably less concentrated, can increase the $O_g(C_{12}H_{22}O_{11})$ of a cell that has been immersed in it for 10 minutes.

SUMMARY

From the preliminary considerations it follows that, to judge the reliability of a plasmolysing agent it is necessary to determine, if the O_g of a cell or tissue remains constant during the period of immersion in the agent.

Table 19

Variations in $O_g(C_{12}H_{22}O_{11})$ of Guard Cells after Immersion in a Potassium Nitrate Solution

(0·a +) = more than 0·a

Serial Number of the Test	$O_g(C_{12}H_{22}O_{11})$ and $O_g(KNO_3)$ of the Tissue	Number of the Cell	$O_g(C_{12}H_{22}O_{11})$ of the Cell	Molarity of the KNO_3 Solution	Final $O_g(C_{12}H_{22}O_{11})$ of the Cell
I	0·625 and 0·45	1	0·6	0·3	0·6 +
		2	0·6	0·3	0·6 +
		3	0·6	0·3	0·6 +
		4	0·6	0·3	0·6 +
		5	0·6	0·3	0·6 +
II	0·625 and 0·45	1	0·6	0·3	0·625 +
		2	0·6	0·3	0·625 +
		3	0·6	0·3	0·625 +
		4	0·6	0·3	0·625 +
III	0·625 and 0·45	1	0·625	0·35	0·65 +
		2	0·625	0·35	0·65 +
		3	0·625	0·35	0·675 +
		4	0·625	0·35	0·675 +
		5	0·625	0·35	0·675 +
		6	0·625	0·35	0·675 +
IV	0.625 and 0·45	1	0·65	0·4	0·7 +
		2	0·65	0·4	0·7 +
		3	0·65	0·4	0·7 +
		4	0·65	0·4	0·7 +
		5	0·65	0·4	0·7 +
		6	0·65	0·4	0·7 +
		7	0·65	0·4	0·7 +
		8	0·65	0·4	0·7 +
V	0·625 and 0·45	1	0·675	0·45	0·725 +
		2	0·675	0·45	0·725 +
		3	0·675	0·45	0·725 +
		4	0·675	0·45	0·725 +

A reliable curve expressing the relation between the molarities of an agent and the equivalent pressures in atmospheres exists for cane sugar but not for potassium nitrate.

Five methods were employed for testing the relative values of cane sugar and potassium nitrate as plasmolysing agents.

The results of the first method indicate that te plasm is permeable for potassium nitrate and that the coefficient of permeability is not constant.

The second method proves that $O_g(KNO_3)$, of the tissues studied, increased, and that $O_g(C_{12}H_{22}O_{11})$ of the same tissues remained constant during the periods of immersion in the proper agents. — The speed of plasmolysis increased with the increment of the molarity up to a certain point, after which it decreased with the increment of the molarity for both agents. — The epidermis and the guard cells deplasmolysed while immersed in potassium nitrate solutions. The speed of deplasmolysis in the guard cells increased with the increment of the molarity up to a certain limit, beyond which it decreased with the increment of the molarity. The speed of deplasmolysis in the epidermal cells, decreased with the increment of the molarity. — The guard cells and the epidermal cells failed to deplasmolyse while immersed in sugar solutions (with one exception, confer table 13).

By means of time records and drawings, it was proven in the third method that $O_g(C_{12}H_{22}O_{11})$ remains constant, while $O_g(KNO_3)$ increased for single cells, during the period of immersion in the respective agents.

In the fourth method it was proven that the immersion in a potassium nitrate solution, of a cell previously plasmolysed in a sugar solution, increased its O_g value. The speed of deplasmolysis in the potassium nitrate solution was independent of the period of immersion in the cane sugar solution.

The results of the fifth method prove that $O_g(C_{12}H_{22}O_{11})$ of a cell is increased by immersion in a hypotonic or isotonic potassium nitrate solution. The increment is most probably due to the penetration of potassium nitrate.

The general conclusion is that the $O_g(C_{12}H_{22}O_{11})$ measurements are reliable, but the $O_g(KNO_3)$ measurements are not reliable, even when the period of immersion in the potassium nitrate solution is limited to the minimum of time required (which can hardly be determined) to plasmolyse the cell or tissue.

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ACKNOWLEDGEMENTS

While attending the University of Fribourg I followed the courses of Professors URSPRUNG, DE GIRARD, REICHENSPERGER, DANIELS, KOWALSKI, GOCKEL, KATHERINER, BISTRZYCKI, ESTREICHER, GLUECKSMANN, DHÉRÉ, GIRARDIN and BAUMHAUER, and Lecturers GYR and GANDOLFI.

I take this occasion to thank them for their personal interest in my formation.

I am particulary grateful to Professor ALFRED URSPRUNG for his guidance and encouragement during the progress of this work, and the touch of personal friendship that has characterized our relations.